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Drinking Water Criteria Document for Hexachlorobenzene (Final Draft)

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DRINKING WATER CRITERIA DOCUMENT FOR HEXACHLOROBENZENE

Prepared for

OFFICE OF DRINKING WATER

Prepared by

Environmental Criteria and
Assessment Office
Cincinnati OH 45268

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The Office of Drinking Water (ODW), U.S. Environmental Protection Agency has prepared a Drinking Water Criteria Document on hexachlorobenzene. This Criteria Document is an extensive review of the following topics:

- Physical and chemical properties of hexachlorobenzene
- Toxicokinetics and human exposure to hexachlorobenzene
- Health Effects of hexachlorobenzene in humans
- Mechanisms of toxicity of hexachlorobenzene
- Quantification of toxicological effects of hexachlorobenzene

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I. SUMMARY

Hexachlorobenzene is imported but not produced commercially in the United States, and occurs as a by-product in the synthesis of nine other chlorinated hydrocarbons; 2-5 million pounds may be generated each year.

Hexachlorobenzene is a colorless crystalline (monoclinic prism) solid with a reported water solubility of 0.005 mg/ℓ at 25°C. In general, hexachlorobenzene has low water solubility, high octanol/water partition coefficient, low vapor pressure at 25°C, low flammability and is photochemically stable. Chemical analysis of hexachlorobenzene in water generally involves solvent extraction followed by a cleanup method being used to produce organic extracts suitable for GC/MS analysis.

The pharmacokinetics of hexachlorobenzene in a number of mammalian species have been studied in detail following oral administration and, to a lesser extent, following intravenous or intraperitoneal injection. No information was present in the available literature on hexachlorobenzene metabolism following inhalation or topical application. Absorption of hexachlorobenzene from the intestinal tract appears to depend on the vehicle used during test material administration. Thus, when hexachlorobenzene is administered in olive oil, ~80% of the dose is absorbed; when it is administered in an aqueous solution, in 1% methyl cellulose or in a crystalline form, relatively little (<20%) is absorbed. Intestinal absorption of hexachlorobenzene occurs primarily through lymphatic channels, with only a minor portion being absorbed into the portal circulation.

Following absorption, hexachlorobenzene is distributed to tissues that have a high lipid content. The adipose tissue accumulates the greatest concentrations of hexachlorobenzene in all species studied, although bone marrow and skin, which contain large amounts of lipids, also accumulate hexachlorobenzene. The adrenal cortex accumulates hexachlorobenzene at concentrations approaching those of fat. Other body constituents (e.g., liver, kidneys, lungs, heart, spleen and blood) generally contain much lower amounts of hexachlorobenzene. Intravenous injection of hexachlorobenzene results in a tissue distribution similar to that seen following oral administration. Hexachlorobenzene is transported via the placenta and is distributed in fetal tissue as indicated by studies in rabbits, rats, mice, mink and ferrets.

Hexachlorobenzene is metabolized slowly into other chlorinated benzenes, chlorinated phenols and other minor metabolites and forms glucuronide and glutathione conjugates. Tissues were found to contain mainly unchanged hexachlorobenzene together with small amounts of metabolites. Similarly, only small amounts of hexachlorobenzene metabolites were detected in feces, whereas most of the metabolites were excreted in the urine together with small amounts of unchanged hexachlorobenzene. There are indications that females produce and excrete more hexachlorobenzene metabolites than do males.

The excretion of hexachlorobenzene from treated animals is slow and occurs mainly through the feces with relatively little being excreted in the urine. It is characterized by an initial rapid phase followed by one or more slow phases. This slow phase of excretion can be enhanced by the administration of mineral oil, paraffin or n-hexadecane. Both biliary and intestinal excretion contribute to fecal excretion. A three-compartment mammalian model has been reported for the behavior of hexachlorobenzene in beagles and rhesus monkeys following i.v. injection of a single dose. Radioactivity was not detected in exhaled air following i.p. injection of ^{14}C-hexachlorobenzene. Hexachlorobenzene has been detected in the milk of nursing mammals.

The acute oral toxicity of hexachlorobenzene has been found to be low with LD_{50} values ranging from 1700-10,000 mg/kg. Subchronic oral toxicity studies with a number of mammalian species indicated a significant increase in liver and kidney weights as well as other organ weights in hexachlorobenzene-treated animals. The livers from hexachlorobenzene-exposed animals have shown histologic changes such as irregular shaped and moderately enlarged liver mitochondria and increases in the size of the centrilobular hepatocytes. Chronic toxicity studies revealed similar effects to those seen in the subchronic studies, plus hexachlorobenzene associated life-shortening and various hepatic and renal pathologies. These subchronic and chronic effects were usually dose-related. Other effects included multiple alopecia and scabbing, together with neurologic effects in rats, mice and dogs. A dose-related histopathologic change in the ovaries of monkeys has also been reported.

Increased porphyrin levels in the liver and in urine have been reported for all species studied except the dog. Hexachlorobenzene was found to cause the accumulation of β-H-steroids which induce porphyrin biosynthesis and to inhibit uroporphyrinogen decarboxylases. The inhibition of uroporphyrinogen decarboxylase appears to be from pentachlorophenol, a hexachlorobenzene metabolite. Indications are that females are more susceptible to hexachlorobenzene-induced porphyria than are males, which may be related to the females estrogen levels and greater hexachlorobenzene metabolism. Hexachlorobenzene was reported to produce a mixed-type induction of cytochromes resembling that produced by a combination of phenobarbital (P-450) and 3,4-benzpyrene (P-448). In addition, the activities of several hepatic microsomal enzymes were found to be induced by hexachlorobenzene.

Hexachlorobenzene did not induce dominant lethal mutations in two studies but was reported to be mutagenic in a yeast, S. cerevisiae, assay at a concentration of 100 ppm. Hexachlorobenzene possessed no detectable levels of mutagenic activity in the Salmonella histidine reversion assay. Chronic feeding studies provide sufficient evidence for the carcinogenicity of hexachlorobenzene in animals since there was an increased incidence of malignant tumors of the liver in two species (haemangioendothelioma in hamsters and hepatocellular carcinoma in rats) as well as reports of hepatoma in mice, rats and hamsters. Hexachlorobenzene given to pregnant mice was found to produce cleft palates and renal agenesis in exposed pups. Certain chemicals were found to alter the toxicity of hexachlorobenzene in mammals, whereas hexachlorobenzene pretreatment was reported to increase CCl_4 toxicity and alter the immune responses of treated animals.

A few epidemiologic studies with occupationally-exposed workers have been reported, together with studies conducted in Turkey and in the United States (i.e., Louisiana) on the general population following accidental exposure to hexachlorobenzene. These studies qualitatively support the toxicity of hexachlorobenzene, but give little dose-response information. Biological monitoring of plasma levels clearly show more hexachlorobenzene in the plasma of exposed compared to nonexposed individuals, although no biologically significant adverse health effects were seen during the observation periods. The exposure of humans to hexachlorobenzene in Turkey during 1955-1959 caused an epidemic of hexachlorobenzene-induced porphyria cutanea tarda (PCT), also known as porphyria turcica, which is manifested by disturbed porphyrin metabolism, cutaneous lesions and hyperpigmentation. The authors estimated that from 0.05-0.2 g/day were ingested. In exposed children under 1 year of age, pink sore was observed as well as 95% mortality in these infants.

Follow-up studies conducted with patients 20-25 years after the onset of porphyria showed that a few subjects still had active porphyria, whereas >50% exhibited hyperpigmentation scarring, as well as other dermatologic, neurologic and skeletal features of hexachlorobenzene toxicity. Hexachlorobenzene residues were also found in the blood, fat and breast milk of some patients.

A correlation was found between hexachlorobenzene levels in blood and the number of years worked in a chlorinated solvents plant. The concentration of urinary uroporphyrins and coproporphyrins ranged from 21-37 and 67-101 μg/ℓ, respectively, for the period between 1974 and 1977. An epidemiologic survey conducted with 86 residents in the vicinity of this chlorinated solvents plant showed elevated hexachlorobenzene residues in plasma. Higher levels of hexachlorobenzene residues were found in males than in females, but these were not associated with race or food consumption.

Quantitative risk assessment for hexachlorobenzene was based on animal data since human data were insufficient. The 1-day HA for a 10 kg child is 0.050 mg/ℓ (0.050 mg/day or 0.005 mg/kg bw/day) and for a 70 kg adult is 0.175 mg/ℓ (0.35 mg/day or 0.005 mg/kg bw/day). The 10-day HA for a 10 kg child is 0.050 mg/ℓ (0.050 mg/day or 0.005 mg/kg bw/day) and for a 70 kg adult is 0.175 mg/ℓ (0.35 mg/day or 0.005 mg/kg bw/day). The lifetime AADI derived from chronic toxicity data for a 70 kg adult is 0.028 mg/ℓ (0.056 mg/day or 0.0008 mg/kg bw/day). The 95% upper bound lifetime cancer risk associated with 1 µg/ℓ of hexachlorobenzene in drinking water is estimated to be 4.9×10^{-5}. Levels of 2.0×10^{-5}, 2.0×10^{-4} and 2.0×10^{-3} mg/ℓ of hexachlorobenzene in drinking water correspond to the 95% upper bound lifetime cancer risks of 10^{-6}, 10^{-5} and 10^{-4}, respectively.

II. GENERAL INFORMATION AND PROPERTIES

Hexachlorobenzene is not manufactured as a commercial product in the United States, but an estimated 2-5 million pounds were produced each year during the synthesis of several chlorinated chemicals (Mumma and Lawless, 1975). Hexachlorobenzene is also an ingredient in a fungicide of which ~200,000 pounds are imported each year (IARC, 1979). Hexachlorobenzene is resistant to biodegradation, accumulates in the biological environment and has been detected in ambient air, drinking and surface water, sediments, cropland and food (U.S. EPA, 1984).

Hexachlorobenzene is a colorless crystalline (monoclinic prisms) solid. Its water solubility was reported as 0.005 mg/ℓ at 25°C (Yalkowsky and Valvani, 1980). Hexachlorobenzene is sparingly soluble in cold alcohol and soluble in benzene, chloroform and ether (NLM, 1979a). Impure commercial preparations may contain pentachlorobenzene (10-81,000 ppm), octachlorodibenzo-p-dioxin (0.05-212 ppm) and octachlorodibenzofuran (0.35-58.3 ppm) (Villeneuve et al., 1974). The chemical structure of hexachlorobenzene is shown in Figure II-1. Synonyms, trade names and identification numbers for hexachlorobenzene are listed in Table II-1.

Some physical properties of hexachlorobenzene are shown in Table II-2. In general., hexachlorobenzene has low water solubility, high octanol/water coefficient, low vapor pressure at 25°C and low flammability. Hexachlorobenzene has been demonstrated to be photochemically stable (Korte et al., 1978; Hustert et al., 1981).

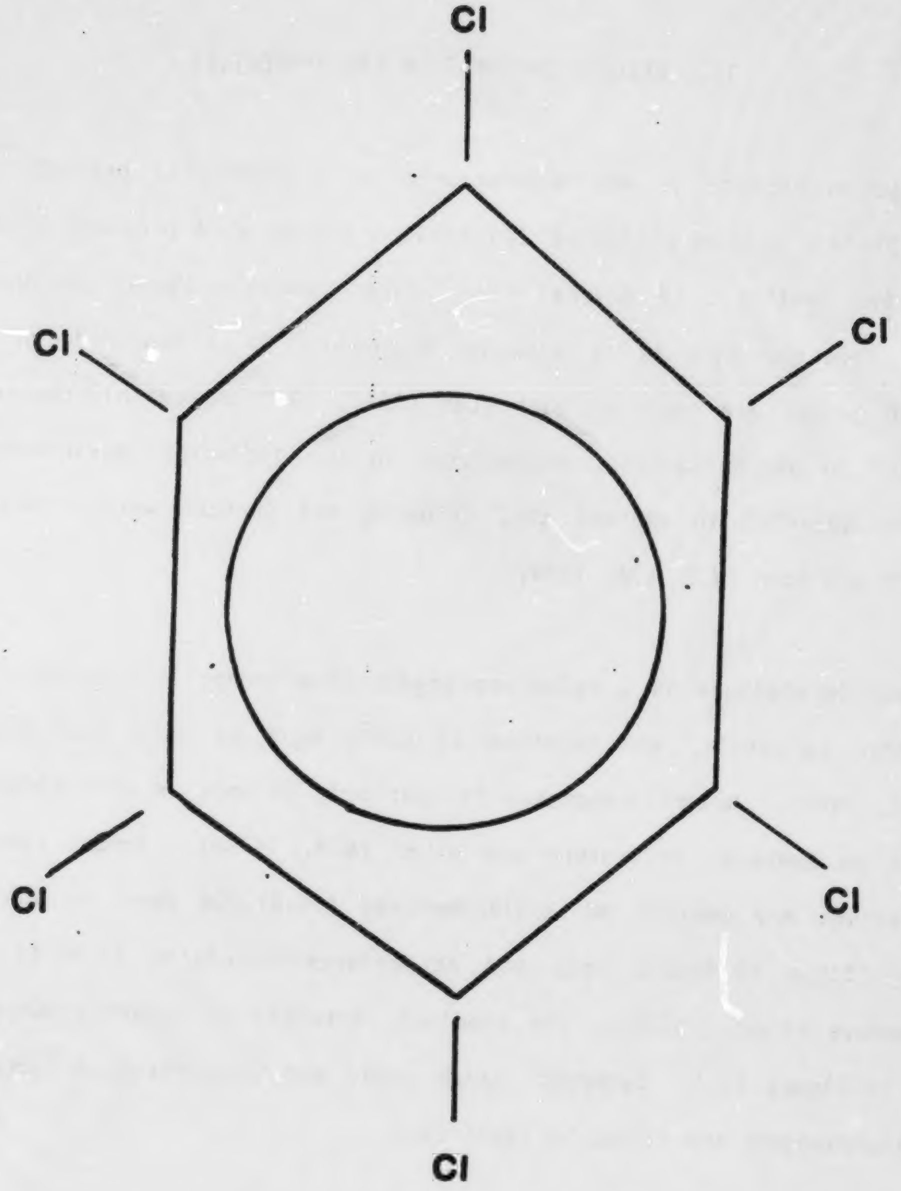
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FIGURE II-1

Chemical Structure of Hexachlorobenzene

TABLE II-1

Synonyms, Trade Names and Identification Numbers of Hexachlorobenzene

Identification Numbers	Synonyms and Trade Names
CAS No. 118-74-1	Esaclorobenzene (Italian)
TSL No. DA2975000	Amatin
EPA Haz Waste No. U127	Anticarie
	Bunt-Cure
	Bunt-No-More
	Co-op Hexa
	Granox NM
	HCB
	HEXA C.B.
	Hexachlorobenzol (German)
	Hexachlorobenzene
	Julin's Carbon Chloride
	No Bunt
	No Bunt 40
	No Bunt 80
	No Bunt Liquid
	Pentachlorophenyl Chloride
	Perchlorobenzene
	Phenyl Perchloryl
	Sanocide
	Smut-Go
	Snieciotox

*Source: National Library of Medicine (NLM), Toxicology Data Bank (TDB)

TABLE II-2

Physical Properties of Hexachlorobenzene[a]

Molecular Weight	284.79
Melting Point	230°C
Boiling Point	322.9°C at 760 mm
Density at 23°C	1.57 g/mℓ
Henry's Law Constant[b] x 10^{-3}	0.12 atm. m^3 mol^{-1}
Log P°[b]	5.8[c]
Water Solubility[d]	0.005 mg/ℓ at 25°C
Flash Point	468°F
Vapor Pressure (mm Hg)	1 at 144.4°C[e] 1.68 x 10^{-5} at 25°F[f] 1.089 x 10^{-5} at 20°C[g]
Specific Vapor Density (air = 1)	9.84[h]

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[a] Data are from the National Library of Medicine (NLM), Toxicology Data Bank (TDB), except as noted.

[b] Mackay et al., 1979

[c] Monsanto, 1978

[d] Yalkowsky and Valvani, 1980

[e] Weast, 1980

[f] Leoni and Darca, 1976

[g] Farmer et al., 1980

[h] Verschueren, 1977

P° = Partition coefficient at 25°C

Chemical Analysis

Chemical analysis of hexachlorobenzene in water generally involves solvent extraction followed by a cleanup method being used to produce organic extracts suitable for GC/MS analysis. The U.S. EPA (1982) (Method 612) has recommeded the use of Florisil column chromatography as a cleanup step before the quantification of the samples by GC with electron capture detector. This recommended method is applicable for the determination of hexachlorobenzene in drinking water and wastewater. The recovery of hexachlorobenzene by this method was found to be 95%.

A method for the determination of hexachlorobenzene in soil and chemical waste disposal site samples has been developed by DeLeon et al. (1980). The procedure involves methane extraction followed by temperature-programmed GC analysis using electron capture detection. Recoveries of samples spiked at the 10, 100 and 300 µg levels were 96.5% (±3.6), 93.1% (±8.1) and 78.0% (±2.6), respectively. The lower detection limit for this method is around 10 µg/g.

III. TOXICOKENETICS

Absorption

Absorption of hexachlcrobenzene from the gut has been studied in detail; however, no information has been found in the available literature on hexachlorobenzene absorption through the lungs or skin. Absorption of hexachlorobenzene from the intestinal tract appears to depend on the solvent vehicle used during test material administration. Thus, when hexachlorobenzene is administered in olive oil, ~80% of the dose is adsorbed; when it is administered in an aqueous solution, in 1% methyl cellulose, or in a solid crystalline form, relatively little (<20%) is absorbed. Intestinal absorption of hexachlorobenzene occurs primarily through lymphatic channels (Iatropoulos et al., 1975), with only a minor portion being absorbed into the portal circulation.

Ingebrigtsen et al. (1981) investigated the absorption of [^{14}C]hexachlorobenzene (10 mg in peanut oil) administered to male, bile-duct-cannulated Wistar rats by gastric catheter. Four days after dosing, 24.8% of the administered ^{14}C had been recovered in the feces, indicating that at least 75% of the administered hexachlorobenzene was absorbed.

Albro and Thomas (1974) studied the absorption of hexachlorobenzene in a squalane/cotton seed oil vehicle by male rats following administration of a single dose by stomach intubation. The results indicated that at doses of 12 and 30 mg/kg, ~82 and 72%, respectively, were absorbed within 96 hours.

Koss and Koransky (1975) compared the absorption rates of [^{14}C]hexachlorobenzene in female Wistar rats following oral administration of olive oil solutions or suspensions in 6% gum arabic in water (4, 20, 50.5, 60 and 180 mg/kg). Approximately 80% of the dose was absorbed from the olive oil solutions; however, only 6% was absorbed from the aqueous suspension.

Similarly, Zabik and Schemmel (1980) found that, when hexachlorobenzene (32 mg/kg/day) was administered in the diet, high-fat (45.3% w/w) diets resulted in greater accumulation of hexachlorobenzene in the tissues and less hexachlorobenzene excreted in the feces than did high-carbohydrate diets (67.7% w/w). The female rats received 32 mg/kg body weight hexachlorobenzene/day for 6, 12 or 18 days. Although this study did not include a control group receiving a balanced diet, the data suggest that high fat diets increase the absorption of hexachlorobenzene.

Sundlof et al. (1982) administered seven consecutive daily oral doses of 10 or 100 mg crystalline hexachlorobenzene/kg body weight to male laboratory beagles. The results from the 100 mg/kg group indicated that hexachlorobenzene can continue to be absorbed from the intestines for up to 1 week following the cessation of dosing.

Bleavins et al. (1982) fed female European ferrets (Mustela putorius furo) a single dose of 57.6 μg hexachlorobenzene (^{14}C-labeled) in 7.5 g of standard mink diet (22% fat) and calculated that 98.5% of the hexachlorobenzene dose was absorbed by the ferrets. They made this calculation based on predicted hexachlorobenzene excretion as extrapolated from this study, and owing to a food passage time in the female ferret of just over 3 hours.

Distribution

Following intestinal absorption, hexachlorobenzene, which is lipophilic, distributes to tissues that are rich in lipid content. The adipose tissue accumulates the greatest concentrations of hexachlorobenzene in all species studied, although bone marrow and skin, which contain large amounts of lipids, also accumulate hexachlorobenzene. The adrenal cortex accumulates hexachlorobenzene at concentrations approaching those of fat. Other tissues (e.g., liver, kidneys, lungs, heart, spleen and blood) generally contain lower amounts of hexachlorobenzene. Intravenous injection of hexachlorobenzene results in a tissue distribution similar to that following oral administration. Hexachlorobenzene is transported via the placenta and is distributed in fetal tissue.

Mehendale et al. (1975) studied the disposition of ^{14}C-hexachlorobenzene by adult male rats following a single oral dose of 5 mg/kg. ^{14}C-Hexachlorobenzene was mixed with arachis oil and administered by stomach intubation at a dose of ~5 mg/kg. The animals were sacrificed 7 days later and the tissues and organs radioassayed. Forty-three percent of the total radioactivity administered was present in fat tissue 7 days after ^{14}C-hexachlorobenzene administration. In addition, muscle and skin tissues each contained ~9% of the radioactivity, whereas the other 12 tissues analyzed contained ~5% combined (Table III-1).

When ^{14}C-hexachlorobenzene was suspended in 1% methyl cellulose and a single oral dose containing 150 μg of hexachlorobenzene was administered to Sprague-Dawley rats, the absorption of ^{14}C-hexachlorobenzene by the walls of the stomach and duodenum 1 hour later was relatively low: ~1.0 and

TABLE III-1

Storage and Excretion of ^{14}C-HCB Administered Orally in Arachis Oil in Rats[a]

Organ or Tissue	Percent of Total Radioactivity Administered
Fat[b]	42.81 ± 6.14
Muscle[c]	9.41 ± 1.17
Skin[d]	8.64 ± 1.21
Liver	3.01 ± 0.23
Small intestine	2.43 ± 0.47
Bone[e]	1.04 ± 0.09
Kidneys	0.76 ± 0.11
Large intestine	0.43 ± 0.08
Stomach	0.36 ± 0.04
Blood	0.24 ± 0.04
Lungs	0.24 ± 0.04
Testes	0.21 ± 0.04
Heart	0.18 ± 0.03
Brain	0.17 ± 0.03
Spleen	0.04 ± 0.002
Total in tissues	70.09 ± 5.48
Excretion	
Feces	16.02 ± 2.31[f]
Urine	0.85 ± 0.13[f]
Gut contents	2.48 ± 0.45
Total recovery	89.44 ± 10.57

[a] Source: Mehendale et al., 1975

[b] Based on 9% body weight as fat

[c] Based on 50% body weight as muscle

[d] Based on 16% body weight as skin

[e] Based on 10% body weight as bone

[f] Cumulative total for 7 days

Adult male rats were given 5 mg/kg of hexachlorobenzene.

HCB = Hexachlorobenzene

0.6 ppm were found in the stomach and duodenum, respectively (Iatropoulos et al., 1975). Increased radioactivity was found in the jejunum and ileum as well as the lymph nodes and adipose tissues 3 hours after administration (Table III-2). Although the radioactivity also increased in the liver and kidneys, this increase was relatively low compared to that found in the lymph nodes and adipose tissue. Moreover, the radioactivity in the liver and kidneys decreased within a 2-day period, whereas the radioactivity in the lymph nodes and fat remained relatively constant. These results indicate that the portal venous transport of hexachlorobenzene to the liver appears to be a minor pathway, whereas the major part of the ingested hexachlorobenzene is absorbed by the lymphatic system in the duodenum and jejuno-ileum and deposited in the fat, bypassing the systemic circulation and the excretory organs.

Knauf and Hobson (1979) investigated the tissue distribution of hexachlorobenzene in six female rhesus monkeys following the gastric administration of daily doses of hexachlorobenzene [0 (one monkey), 8 (one monkey), 32 (one monkey), 64 (one monkey), or 128 (two monkeys) mg/kg/day] in 1% methyl cellulose for a period of 60 days. The highest concentrations of hexachlorobenzene were located in tissues with high lipid content. Tissue levels correlated more with body fat content than with dose, with the monkey that had the least adipose tissue producing the highest nonfat tissue and serum values (Table III-3).

The highest levels of hexachlorobenzene residues were found in fat tissue (215-930 ppm) and bone marrow (175-1700 ppm), and selectively higher levels were found in the adrenal cortex (30-325 ppm) than in the adrenal

TABLE III-2

Tissue Concentration (ppm) of ^{14}C-Hexachlorobenzene[a] and Its Metabolites in Sprague-Dawley Rats[b]

Tissue	Time (hours)									
	1		3		5		12		48	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Stomach	0.6	1.6	0.8	1.0	1.1	0.5	0.1	0.1	0.1	0.1
Duodenum	0.6	0.6	1.4	1.0	0.2	0.3	0.1	0.1	0.1	0.1
Jejuno-Ileum	0.1	0.2	0.6	0.8	1.0	0.3	0.3	0.3	0.2	0.1
Cecum	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.1
Colon	0.1	0.1	0.1	0.2	0.4	0.1	0.1	0.1	0.2	0.1
Liver	0.1	0.4	0.5	0.5	0.2	0.3	0.2	0.2	0.1	0.2
Mesenteric lymph node	0.1	0.6	0.4	1.3	2.0	1.0	1.5	1.0	1.9	2.1
Adipose tissue	0.1	0.2	1.7	1.2	2.3	1.5	1.3	1.1	2.6	2.7
Kidneys	0.1	0.2	0.4	0.3	0.5	0.2	0.2	0.1	0.2	0.1
Lungs	0.1	0.3	0.3	0.4	0.2	0.2	0.1	0.1	0.1	0.2

[a] 150 μg hexachlorobenzene was administered by stomach tube suspended in 1% methyl cellulose.

[b] Source: Iatropoulos et al., 1975

TABLE III-3

Tissue Levels of HCB (ppm) in Adult Female Rhesus Monkeys[a,b]

Monkey No.	613[c]	618[d]	627[e]	817	1163	1826
Dose (mg/kg/day)	128	128	64	32	8	0
Body fat	930	215	540	250	580	1.1
Bone marrow	460	175	1700	255	350	1.6
Adrenal cortex	150	30	325	90	50	0.1
Adrenal medulla	12	9	285	35	4	<0.1
Liver	20	50	365	40	30	<0.1
Kidney	18	19	258	11	3	<0.1
Brain	25	19	108	12	8	<0.1
Ovaries	6	23	133	3	1	<0.1
Muscle	4	21	24	7	2	<0.1
Serum	2.5	1.5	11.0	0.5	3.3	<0.1

[a] Source: Knauf and Hobson, 1979

[b] HCB was administered daily for 60 days in 1% methylcellulose (orally)

[c] Monkey was small and slight

[d] Monkey was obese

[e] Monkey had very little adipose tissue

HCB = Hexachlorobenzene

medulla (4-285 ppm). Residues in semen, muscle, ovaries, brain, kidneys and liver were relatively much lower (0.5-258 ppm).

Engst et al. (1976) reported the administration by gavage of 8 mg/kg of hexachlorobenzene in 1 mℓ of sunflower oil to male Wistar rats for a duration of 19 days. The animals were then sacrificed, and the liver, kidneys, adrenals, heart, spleen and intestinal fat were analyzed for hexachlorobenzene residues. The following results were reported: fat tissue, 82 μg/g; muscle, 17 μg/g; liver, 125 μg total; kidneys total 21 μg each; spleen total 9 μg; heart total 1.5 μg; and adrenals total 0.5 μg each. High levels of hexachlorobenzene residues in fat tissues also have been reported for rats receiving 50.0 mg/kg (177 μmoles/kg) of hexachlorobenzene every second day for 10 weeks (Koss et al., 1980b).

Szymczynski and Waliszewski (1981) analyzed human semen and testicular and fat tissues, and identified several chlorinated pesticides that included hexachlorobenzene. The compound was not detected in testicular tissue, but was present in semen and fat tissues at concentrations of 0.001 and 0.128 μg/g, respectively. Similarly, hexachlorobenzene was one of several chlorinated compounds found in semen collected in 1979 from 132 college students (Dougherty et al., 1981).

Sundlof et al. (1982) studied the distribution of ^{14}C-hexachlorobenzene or unlabeled hexachlorobenzene in male beagles following a single intravenous dose of 1 mg/kg in olive oil. Two dogs each were sacrificed after 2, 4, 8, 16, 32 and 48 hours and after 12 weeks; hexachlorobenzene concentrations were determined in 16 tissues and organs as well as in the blood (Table III-4). Two hours after dosing, the highest concentration was found in the lungs (36.14 ppm). This was considered to be a property of the injection vehicle rather than a property of hexachlorobenzene per se. That is, it was believed that the olive oil vehicle formed microemboli in the blood which became trapped in the capillaries of the lung. Residue levels in the lungs then dropped (4.4 ppm), and a concurrent increase in hexachlorobenzene was detected in fat tissues (10.32 ppm in subcutaneous, perirenal and mesenteric fat) 4 hours postinjection. Residues in all tissues, organs and blood declined during the 48 hours postinjection except for fat tissue, which remained constant. Twelve weeks after dosing, tissue concentrations were very low in all tissues, including fat (>0.01-0.46 ppm), indicating significant excretion of the compound by that time.

Yang et al. (1978) studied the distribution of hexachlorobenzene in male Sprague-Dawley rats and female rhesus monkeys following intravenous injection of ^{14}C-hexachlorobenzene in 1,2-propanediol:plasma (1:8). Rats received 0.1 mg of ^{14}C-hexachlorobenzene and then were replaced in metabolic cages for 48 hours before sacrifice. About 0.2 and 1.0% of the administered dose was excreted in the urine and feces, respectively. No radioactivity was exhaled from the animals. Over 20 tissues from the rats were analyzed and all were found to contain radioactivity. The highest levels were in fat (~3 μg/g of tissue). The adrenal glands also contained a relatively high level of radioactivity, whereas the other tissues contained much lower levels, generally in the range of 1/12 to 1/300 of those in fat tissue.

TABLE III-4

HCB Concentrations in Tissues of Male Beagles Receiving Single Intravenous Doses of 1 mg/kg bw in Olive Oil*

Tissue	HCB Concentration (ppm) Time Interval After Dosing 2 hours	 48 hours	 12 weeks
Lungs	36.14	0.08	<0.01
Adrenals	2.82	0.38	0.06
Subcutaneous fat	1.14	3.38	0.37
Perirenal fat	1.00	3.24	0.46
Mesenteric fat	0.56	2.40	0.41
Spleen	0.54	0.01	<0.01
Liver	0.51	0.04	0.02
Thyroid	0.37	NR	0.02
Heart	0.28	0.04	0.01
Kidneys	0.18	0.02	0.01
Stomach	0.18	0.36	0.01
Pancreas	0.17	0.06	0.07
Brain	0.15	0.02	0.02
Duodenum	0.12	0.02	0.02
Colon	0.12	0.01	<0.01
Small intestine	0.07	0.02	0.01
Blood	0.07	0.03	0.01

*Source: Sundlof et al., 1982

NR = Not reported

HCB = Hexachlorobenzene

The tissue distribution of ^{14}C-hexachlorobenzene in rhesus monkeys was determined in individual animals 100 days, 6 months and 1 year after intravenous injection of ^{14}C-hexachlorobenzene at 0.38, 0.32 and 0.22 mg/kg, respectively. The results again indicated that the highest levels were present in fat (6069 ng/g on day 100 and 828 ng/g on day 365) and bone marrow (1638 ng/g on day 100 and 373 ng/g on day 365) among the 30 tissues analyzed in all three monkeys. The adrenal glands contained ~1/6 to 1/8 of the levels present in fat, whereas the other tissues contained radioactivity levels ranging between 1/10 to <1/800 of those in fat.

The transplacental transfer of hexachlorobenzene from pregnant mice, rats and rabbits has also been reported. Brandt et al. (1980) conducted a qualitative study on the distribution of ^{14}C-hexachlorobenzene and several of its sulfur-containing metabolites in pregnant mice. The mice were injected i.v. and sacrificed at intervals ranging between 20 minutes and 32 days after injection. The animals were frozen, sectioned and submitted to autoradiography. The autoradiograms showed a strong uptake of hexachlorobenzene in the adipose tissues. This hexachlorobenzene was found to persist in the adipose tissues for more than 1 month after the administration. Radioactive hexachlorobenzene was also found to penetrate the placenta, resulting in the blood and liver concentrations in the fetus which appeared to equal those of the dams.

Villeneuve and Hierlihy (1975) studied the placental transfer of hexachlorobenzene in Wistar rats and reported that hexachlorobenzene crosses the placenta and accumulates in the fetus in a dose-dependent manner. The females were dosed orally daily (5, 10, 20, 40 and 80 mg/kg) from gestation day 6-16 and then sacrificed on day 22. Only liver, brain and whole fetus residue levels were determined in this study. Fetal liver residues (1.8-35.8 μg/g) were much lower than those of the dams (9.3-86.0 μg/g). The fetal brain and whole fetus levels were 1.1-17.5 μg/g and 1.5-18.9 μg/g, respectively.

Villeneuve et al. (1974) also reported that the transplacental transport of hexachlorobenzene in New Zealand rabbits was dose-dependent. Rabbits were mated and then treated orally with hexachlorobenzene from days 1-27 with subtoxic doses of 0, 0.1, 1.0 or 10 mg/kg. On day 28 the dams were killed for fetal and maternal tissue analysis for hexachlorobenzene. In dams, the hexachlorobenzene residue concentrations were highest in fat, followed by the liver, heart, kidneys, brain, lung, spleen and plasma. Hexachlorobenzene residues were higher in the fetal liver than in the maternal liver.

Courtney et al. (1976) reported on the distribution of hexachlorobenzene (assayed 90.4% hexachlorobenzene and 9.6% pentachlorobenzene) administered via oral intubation on days 7-11 of gestation at a dose of 50 mg/kg/day in a corn oil acetone mix to five pregnant and two non-pregnant CD-1 mice. They found there were no remarkable differences in the hexachlorobenzene tissue levels between the pregnant and non-pregnant animals sampled at day 12 of pregnancy. The levels of pentachlorobenzene in sampled tissues were low as compared to the very high hexachlorobenzene levels detected in the thymus, skin, fat and urinary bladder. No detectable levels of hexachlorobenzene or pentachlorobenzene were found in the control mice.

Courtney et al. (1979) studied the tissue distribution of hexachlorobenzene in the maternal and fetal tissues of CD rats and CD-1 mice and reported that placentas and fetuses of both species demonstrated a dose-dependent relationship for hexachlorobenzene residues, with levels in the fetuses being higher than those in their corresponding placentas. The dams were treated via oral intubation with single or multiple oral doses (10, 50 or 100 mg/kg in corn oil) at different periods during gestation. The hexachlorobenzene concentrations in mice and rat fetuses at mid-gestation were very similar. In mice, multiple low doses of hexachlorobenzene resulted in higher concentrations of hexachlorobenzene in maternal and fetal tissues than single doses of equivalent total doses. In another study, Courtney and Andrews (1979) reported that in mice the fetus could be exposed to hexachlorobenzene from maternal body burdens, established before fetal implantation, and was not limited to maternal exposure during the postimplantation gestation.

Bleavins et al. (1982) studied the tissue distribution and transfer of a single dose of hexachlorobenzene given to female European ferrets (_Mustela putorius furo_). They gave a single 57.6 µg hexachlorobenzene (^{14}C-labeled) dose to each of three bred and five non-bred ferrets, in 7.5 g of standard mink diet (22.2% fat). The dosed ferrets and offspring were observed for 5 weeks after the kits were born, at which time they were killed and tissue ^{14}C-hexachlorobenzene levels were determined (Table III-5). One ferret kit per litter was also collected at birth and at weeks 1, 2, 3 and 4 for whole body residue determinations (Table III-6). These results indicate that nursing mothers can significantly reduce their body burdens of hexachlorobenzene, when compared to unbred female counterparts, by transferring a large amount of the hexachlorobenzene to their offspring.

TABLE III-5

Mean (±SE) Hexachlorobenzene Radioactivity (dpm/g) of Selected European Ferret Tissues[a,b]

Tissues	Group I (n=3)	Group II (n=5)	Kits[c] (n=3)
Blood	49 ± 34.6[d]	166 ± 26.8	--
Subcutaneous fat	4472 ± 780.5[e]	19,525 ± 1503.9	11,678 ± 712.4[f]
Visceral fat	4429 ± 867.6[e]	19,704 ± 1666.0	--
Muscle	53 ± 14.4[d]	384 ± 64.0	561 ± 204.8
Heart	34 ± 9.2[d]	310 ± 56.8	--
Kidney	105 ± 31.1[e]	611 ± 80.4	209 ± 37.2
Spleen	13 ± 7.5[e]	180 ± 24.8	--
Liver	248 ± 68.9[e]	1,445 ± 145.2	1,420 ± 185.6[g]
Lung	1 ± 0.3[e]	241 ± 18.4	--
Brain	61 ± 30.0[e]	395 ± 48.5	130 ± 29.4

[a] Source: Bleavins et al., 1982

[b] at 62 days postdosing from adult bred (group I) and unbred (group II) female ferrets exposed to a single 57.6 µg dose of ^{14}C-labeled hexachlorobenzene and from offspring born to the bred females.

[c] Kit tissues, from 5-week-old offspring, were contrasted only with maternal (group I) tissues.

[d] Significantly different ($p<0.05$) from group II tissue of the same type.

[e] Significantly different ($p<0.01$) from group II tissue of the same type.

[f] Significantly different from maternal tissue (group I) at $p<0.01$.

[g] Significantly different from maternal tissue (group I) at $p<0.05$.

HCB = Hexachlorobenzene

TABLE III-6

Mean (±SE) HCB Radioactivity (dpm x 10^3) of European Ferret Kits[a,b]

Measure	Number	Weeks Postpartum					
		0	1	2	3	4	5
Per gram of kit	3	3.0 ± 0.19	2.7 ± 0.57	4.3 ± 0.67	3.9 ± 0.73	3.5 ± 0.50	2.7 ± 0.14
Per whole kit	3	25.1 ± 1.43	76.7 ± 14.35	311.4 ± 63.39	492.5 ± 92.22	672.8 ± 117.63	805.7 ± 54.25
Increase over previous week		--	51.6	234.7	181.1	180.3	132.8
Milk (per ml)	3	--	--	6.1 ± 0.66	2.9 ± 0.45	1.8 ± 0.17	0.8 ± 0.20

[a] Source: Bleavins et al., 1982

[b] Born to female ferrets exposed to a single dose of ^{14}C-labeled hexachlorobenzene and the milk produced by those dams

HCB = Hexachlorobenzene

The mothers' milk contaminated with hexachlorobenzene seems to be a large contributor to the kits' body burdens with a reported milk to placental exposure ratio of 31:1. The distribution of hexachlorobenzene in ferrets follows similar trends, as observed in the other mammals, where the highest hexachlorobenzene levels were found in the lipid rich tissues.

The transfer of hexachlorobenzene to nursing infant rhesus monkeys from lactating mothers receiving via oral intubation 64 mg/kg/day hexachlorobenzene suspended on 1% methyl cellulose for 60 days was reported by Bailey et al. (1980). Milk concentrations were on the average 17-fold higher than maternal serum levels, whereas infant serum levels were about 2- to 5-fold higher than serum levels of their mothers. Similarly, the infants had higher tissue residues than their mothers and hexachlorobenzene was concentrated in the infant fat, bone marrow, adrenals and lymph nodes.

Hexachlorobenzene residues also have been reported in human fat in the United Kingdom (Abbott et al., 1981, Japan (Curley et al., 1973), and Australia (Brady and Siyali, 1972) and in human milk collected in Sweden (Westoo and Noren, 1978; Hofvander et al., 1981), Canada (Mes and Davies, 1979), Norway (Bakken and Seip, 1976; Skaare, 1981), and Hawaii (Takahashi et al., 1981).

Metabolism

The metabolism of hexachlorobenzene has been studied in male and female rats following oral administration, rhesus monkeys and beagles following i.v. injection, and rabbits following i.p. injection (Renner, 1981). Hexachlorobenzene is metabolized slowly into other lower chlorinated benzenes, chlorinated phenols and other minor metabolites and forms glucuronide and g lutathione conjugates. Tissues were found to contain mainly unchanged hexachlorobenzene together with small amounts of metabolites. Similarly, only small amounts of hexachlorobenzene metabolites were detected in feces, whereas most of the metabolites were excreted in the urine together with small amounts of unchanged hexachlorobenzene.

Mehendale et al. (1975) studied the metabolism of hexachlorobenzene in male Sprague-Dawley rats 7 days after oral intubation administration of a single 5 mg/kg dose in arachis oil. The fat, liver, intestines, kidneys, lungs and brain were found to contain hexachlorobenzene primarily, along with trace amounts of other chlorinated benzenes. Analysis of these chlorinated benzenes suggested the presence of pentachlorophenol, 2,4,5-trichlorophenol, pentachlorobenzene and the tetrachlorobenzenes. Extraction and analysis of fecal radioactivity, which accounted for 16% of the dose, did not reveal the presence of metabolites. Although urine contained only 0.85% of the administered radioactivity, it provided the only evidence of hexachlorobenzene metabolite excretion. Several unidentified metabolites were evident following thin-layer chromatography (TLC) separation of urine, in addition to 2,4,5-trichlorophenol, pentachlorophenol and one spot was reported to contain a mixture of chlorinated benzenes.

In vitro metabolism studies with homogenates of the liver, lungs, kidneys and small intestines produced trace amounts of chlorobenzene metabolites when incubated with [^{14}C]-hexachlorobenzene in the presence or absence of added cofactors. Liver microsomal preparations produced amounts of one or more chlorophenols when fortified with NADPH; in the presence of UDPGA, pentachlorophenol was reported to form the glucuronide conjugate.

Fortification of kidney homogenates with glutathione resulted in the appearance of unextractable radioactivity in the aqueous phases, indicating that glutathione conjugates of polar hexachlorobenzene metabolites might also be formed (Mehendale et al., 1975).

The metabolism of hexachlorobenzene in male and female Sprague-Dawley rats each receiving nine oral doses of 85.6 mg/kg hexachlorobenzene (99.7% pure) in arachis oil over a period of 1 month was reported by Richter et al. (1981). The animals were sacrificed 3, 24 and 52 days after the last dose, and various tissues were analyzed for hexachlorobenzene and its metabolites by CDE/GLC and GLC/MS. In addition to hexachlorobenzene, the following metabolites were also detected: pentachlorobenzene (PCB), pentachlorophenol (PCP), pentachlorothiophenol (PCTP) and 2,3,4,6- and 2,3,5,6-tetrachlorophenol (TCP). The results reported for the liver and kidneys for day 3 indicated that the livers of the females contained significantly more PCTP, a derivative of a glutathione conjugate, than those of the males (Table III-7). However, it is not known whether this increase is due to a higher rate of PCTP production or to a lower rate of elimination.

Rizzardini and Smith (1982) investigated the sex differences in hexachlorobenzene metabolism in young F344/N rats who had been intubated every other day for 103 days with 14 mg/kg hexachlorobenzene (analytical grade) dissolved in arachis oil. Three hexachlorobenzene metabolites were analyzed for: pentachlorobenzene, pentachlorothiophenol and 2,3,5,6-tetrachlorobenzene-1,4-diol, and all three were found to be produced in larger concentrations in the female rats during the first 10 weeks of hexachlorobenzene treatment. The greater quantities of hexachlorobenzene metabolites being formed in female rats was believed due to their body estrogen levels.

TABLE III-7

Concentrations of HCB and its Metabolites (mg/kg) in the Liver and Kidneys of Male and Female Rats[a,b]

Tissue/Sex	HCB	PCB	PCP	PCTP	TCP
Liver					
Males	192	0.05	3.16	0.23	0.02
Females	147[c]	0.03[c]	2.12[c]	0.36[c]	0.04[c]
Kidneys					
Males	127	0.05	5.79	0.24	0.09
Females	111	0.01	3.69	0.10	0.08

[a] Source: Richter et al., 1981

[b] Determined 3 days after the last of nine oral doses of 85.6 mg/kg HCB given within 1 month in arachis oil

[c] Statistically significant from males ($p<0.05$)

HCB = Hexachlorobenzene; PCB = pentachlorobenzene; PCP = pentachlorophenol; PCTP = pentachlorothiophenol; TCP = 2,3,5,6-tetrachlorophenol

Engst et al. (1976) detected several urinary metabolites in male Wistar rats receiving by gavage 8 mg/kg of hexachlorobenzene daily dissolved in sunflower oil for 19 days. The results of this study were presented qualitatively, and the authors reported that the major metabolic route for hexachlorobenzene was to pentachlorophenol. In addition, the feces contained mainly unchanged hexachlorobenzene together with traces of pentachlorobenzene.

Koss et al. (1976) investigated the metabolism of hexachlorobenzene in female Wistar rats given 2-3 i.p. doses of [^{14}C]hexachlorobenzene (260 or 390 mg/kg total dose). At the end of 4 weeks, 7% of the administered radioactivity was excreted in the urine, with >90% of this amount contained in the major metabolites (pentachlorophenol, tetrachlorohydroquinone, and pentachlorothiophenol). An isomer of tetrachlorothiophenol was detected as a minor urinary metabolite. Twenty-seven percent of the administered radioactivity was excreted in the feces, of which 70% was identified as unchanged hexachlorobenzene. Only pentachlorophenol and pentachlorothiophenol were identified as fecal metabolites of hexachlorobenzene. In the tissues of the animals, only pentachlorophenol was detected in measurable quantities, accounting for 10% of the radioactivity in blood and <0.1% in body fat. Total radioactivity contained in the metabolites detected in the animal bodies and excreted at the end of the 4 weeks accounted for 16% of the administered radioactivity.

In follow-up studies, Koss et al. (1978a) compared the formation of hexachlorobenzene metabolites in rats, mice, guinea pigs, Japanese quail, laying hens and rainbow trout. The only metabolites detected were pentachlorophenol, tetrachlorohydroquinone and pentachlorothiophenol; however, the species tested differed greatly in their ability to metabolize hexachlorobenzene (Table III-8).

Gas-liquid chromatography of urine, bile and fecal extracts from male beagle dogs receiving a single i.v. injection of ^{14}C-hexachlorobenzene at 1 mg/kg revealed that 96% of the fecal radioactivity occurred as the parent compound. Hexachlorobenzene accounted for 4% of the biliary radioactivity, but no parent compound was detected in urine (Sundlof et al., 1982).

Kohli et al. (1976) studied the metabolism of several chlorinated benzenes, including hexachlorobenzene, in rabbits following i.p. injection. The urine was collected for 10 days after injection and analyzed for metabolites following extraction and gas-liquid chromatography, but no hexachlorobenzene metabolites were found in the urine.

Excretion

The excretion of hexachlorobenzene from treated animals is slow and occurs mainly through the feces, with relatively little being excreted in the urine. It is characterized by an initial rapid phase followed by a very slow phase. This slow phase of excretion can be enhanced by the administration of mineral oil, paraffin and n-hexadecane. Both biliary and intestinal excretion contribute to fecal excretion. A three-compartment mammalian model has been reported for the behavior of hexachlorobenzene in beagles and rhesus monkeys following i.v. injection of a single dose. Radioactivity was not detected in exhaled air following i.p. injection of ^{14}C-hexachlorobenzene.

TABLE III-8

Hexachlorobenzene and Its Major Metabolites in the Excreta of Different Animal Species[a]

Species[b]	Total Dose[c] (mMol/kg)	Total Amount of Substances			
		HCB	PCP	TCH	PCTP
Rat	0.92	6.1[d]	2.0	0.4	1.8
Mouse	0.92	2.6	0.3	0.1	ND
Guinea pig	0.92	1.8	0.9	0.2	0.5
Japanese quail	2.76	7.5	trace	trace	3.2
Laying hen	0.92	0.6	0.1	0.07	0.04
Rainbow trout	2.76	1.8	0.4	ND	ND

[a] Source: Koss et al., 1978a

[b] 2-3 animals were used per each species investigated

[c] Hexachlorobenzene was dissolved in oil and administered intraperitoneally

[d] Figures are given in μMol/kg bw/day

ND = Not detected. The lower detection limit of the metabolites was determined to be 0.03 nMol/mℓ urine or g feces.

HCB = Hexachlorobenzene; PCP = pentachlorophenol; TCH = tetrachlorohydroquinone; PCTP = pentachlorothiophenol

Studies conducted by Mehendale et al. (1975) with rats receiving a single oral dose indicated that only 16.0 and 0.85% were excreted in the feces and urine, respectively, 7 days after treatment (see Table III-1). Ingebrigtsen et al. (1981) reported that 4 days after intragastric administration of ^{14}C-hexachlorobenzene, a total of 24.8 and 2.1% of the administered radioactivity were recovered in the feces and urine, respectively. In addition, an average of 3.6% of the dose was recovered in the bile of bile-duct-cannulated rats within 48 hours after dosing. Of the radioactivity excreted in the bile, only 2% was unchanged hexachlorobenzene, 1.8% was pentachlorobenzene, 24% was pentachlorophenol and ~72% was unidentified metabolites.

Rozman et al. (1977) studied the excretion of hexachlorobenzene in female rhesus monkeys receiving 110 μg ^{14}C-hexachlorobenzene/day/monkey via diet for 15 months. The excretion and storage patterns showed a very slow approach to a saturation level, indicating a high tendency for hexachlorobenzene accumulation in rhesus monkeys. A total of 5.8 and 3.6% of the administered dose was excreted in the urine of male and female monkeys, respectively, after 15 months, of which 50% was pentachlorophenol, 25% pentachlorobenzene and the remaining 25% consisting of unidentified metabolites with varying amounts of hexachlorobenzene. A total of 47.9 and 27.5% of the dose was present in the feces of male and female monkeys, respectively, of which 99% was hexachlorobenzene.

Koszo et al. (1978) administered hexachlorobenzene (0.2% in the diet) to young male and female Wistar rats for as long as 200 days and measured the accumulation of hexachlorobenzene in the liver and fatty tissue and the excretion of hexachlorobenzene and pentachlorophenol in the urine and feces. The concentration of hexachlorobenzene in the liver and fat increased steadily throughout the treatment period. Pentachlorophenol appeared in both the urine and feces in increasing amounts throughout the treatment period, with the excretion of other apolar and polar products being markedly enhanced after 5-6 weeks.

Rizzardini and Smith (1982) investigated the sex differences in hexachlorobenzene metabolism and excretion of hexachlorobenzene metabolites in young F344/N rats. These rats were intubated with 14 mg/kg analytical grade hexachlorobenzene dissolved in arachis oil every other day for 103 days and were analyzed for the three main hexachlorobenzene metabolites, pentachlorophenol, pentachlorothiophenol and 2,3,5,6-tetrachlorobenzene-1,4-diol, in urine and feces. Results indicated that the combined urinary excretion of metabolites was greater in the female rats, especially during the first 10 weeks, with pentachlorothiophenol being particularly high in the females. No wide variations between the sexes were seen in the analyzed feces hexachlorobenzene metabolites after 103 days of treatment. Combined urine and feces excretion of metabolites at the end of the study were found not to be significantly different between males (2291±116 nmole/ 24 hours/kg) and females (2425±182 nmole/24 hours/kg). It was stated, though, that the total excretion of pentachlorothiophenol was always significantly higher in the female rats.

Koss and Koransky (1975) studied the metabolism of hexachlorobenzene in rats when the compound was orally administered in an aqueous suspension or in olive oil. The animals received different amounts of ^{14}C-hexachlorobenzene in a single dose, and the feces and urine were collected at varying time intervals and radioassayed. When administered in water, hexachlorobenzene was not readily absorbed; 76-97% of the dose was excreted in the feces, and <0.1-0.4% was excreted in the urine 1 day after administration. When administered in oil, only 45-46% of the dose was excreted in the feces and 2.1-3.8% was excreted in the urine after 14 days of treatment. Rats receiving 4 mg/kg of ^{14}C-hexachlorobenzene administered i.p. excreted a total of 5 and 34% of the dose in the urine and feces, respectively, within 14 days. About 4 and 80% of the excreted radioactivity in the urine and feces, respectively, was unchanged hexachlorobenzene. Animals injected i.p. with 50.5 mg/kg [^{14}C]hexachlorobenzene released no radioactivity in exhaled air (Koss and Koransky, 1975).

Rozman et al. (1981) reported that administration of mineral oil or n-hexadecane to female Sprague-Dawley rats or male or female rhesus monkeys who were pretreated with ^{14}C-hexachlorobenzene enhanced the fecal elimination of ^{14}C-hexachlorobenzene. All animals were administered ^{14}C-hexachlorobenzene (100 mg/kg) in 1% methyl cellulose as a single dose oral intubation except for one monkey that received three consecutive daily doses and two monkeys that received ^{14}C-hexachlorobenzene (0.11 mg/kg) in sugar pellets daily for 750 consecutive days. Aliphatic hydrocarbons were administered to the treated animals 11-40 days after hexachlorobenzene treatment. When mineral oil was added to the diet of the rhesus monkeys, fecal excretion of hexachlorobenzene was enhanced 6- to 9-fold. Similarly, dietary administration of hexadecane resulted in the same increase in fecal excretion of hexachlorobenzene in both the rhesus monkeys and rats. Residue analyses indicated an enhanced depletion of hexachlorobenzene from blood and of stored hexachlorobenzene from adipose tissue. Enhanced fecal excretion of hexachlorobenzene as a result of dietary administration of aliphatic hydrocarbons was primarily due to increased hexachlorobenzene elimination in the large intestine.

Richter and Schafer (1981) studied the intestinal excretion of hexachlorobenzene in male Sprague-Dawley rats using the pendular perfusion method. The animals were injected i.p. with hexachlorobenzene at 100 mg/kg and, 1 and 4 weeks after treatment, various parts of the intestines were perfused with paraffin or squalane for 5 hours. The largest amount of hexachlorobenzene excreted was into the jejunum followed by the ileum and colon. The ratios of total hexachlorobenzene excreted during paraffin treatment were: jejunum/ileum = 1.26 and jejunum/colon = 2.43. The authors concluded that these results indicate the importance of intestinal excretion in the elimination of hexachlorobenzene, and that paraffin treatment can be one of the measures by which a long-term stimulation of hexachlorobenzene excretion can be achieved.

Beagle dogs receiving a single i.v. dose of 1 mg/kg excreted 44 and <6% of the dose in the feces and urine, respectively, during a 12-week period (Sundlof et al., 1982). Both biliary and intestinal excretion contributed to fecal excretion; however, the data indicated that biliary excretion was the major contributor to fecal excretion. A computer-assisted pharmacokinetic analysis of blood, urine and fecal radioactivity levels during a 12-week period suggested a three-compartment model for the behavior of hexachlorobenzene in beagles. The biological half-life values were calculated for the three dogs used and ranged from 6 weeks to 3 years.

Yang et al. (1978) reported that the elimination rate of hexachlorobenzene from male Sprague-Dawley rats and rhesus monkeys injected i.v. with hexachlorobenzene was slow because hexachlorobenzene is stored in the fat tissue. The major route of excretion for the radiolabel in treated monkeys was via the feces. About 17.1, 8.8 and 28.2% of the dose was excreted in the feces after 100 days, 6 months and 1 year, respectively, after treatment of individual monkeys, with ~90% of the radioactivity determined to be unchanged ^{14}C-hexachlorobenzene. The cumulative urinary excretion of hexachlorobenzene metabolites was determined to be 1.6% of the administered dose after 1 year. An open system, three-compartment mammillary model was found to fit the data for plasma, fecal and metabolized hexachlorobenzene in the rhesus monkey.

Koss et al. (1983) administered 100 mg/kg hexachlorobenzene in olive oil every other day, via stomach tube, to female Wistar rats for a period of 6 weeks and then observed the rats for an additional 18 months. At cessation of hexachlorobenzene treatment they tried to assess the biological half-life of hexachlorobenzene and determined a value of 8 days for the start of the elimination phase, a value of 10 weeks when assessed 3 months later, and finally a value of 1.5 years after 12 months. The authors then concluded that it is not possible to establish a valid biological half-life for the total elimination phase of hexachlorobenzene in rats.

Bleavins et al. (1982) studied the excretion and transfer of hexachlorobenzene given to female European ferrets (Mustela putorius furo). Three bred and five non-bred female ferrets were each given a single dose of 57.6 μg ^{14}C-hexachlorobenzene in 7.5 g of standard mink diet (22.2% fat).

The investigators indicated that there were no significant differences in the excretion of hexachlorobenzene metabolites, between bred and non-bred groups, in urine for the entire 8-week study period or in feces during the beginning of the study. The observed fecal excretion during the middle weeks to the end of the study showed a leveling of the cumulative fecal excretion in the bred females and a continued increase in fecal excretion in the non-bred female ferrets, although it was stated that this difference was not statistically significant. Excretion of hexachlorobenzene or metabolites in the milk was found to be an important route of excretion for lactating females, ~20.3% of the initial dose was eliminated by the fifth week of lactation, and found to be a very important route of exposure to nursing offspring. The importance of placental transfer and milk excretion is further presented by observing the time required for 50% of the initial hexachlorobenzene dose to be excreted. The bred females required 32 days to excrete 50% while 41 days was required for the unbred females.

Summary

The pharmacokinetics of hexachlorobenzene in a number of mammalian species have been studied in detail following oral administration and, to a lesser extent, following i.v. or i.p. injection. No information was present in the available literature on hexachlorobenzene metabolism following inhalation or topical application. Absorption of hexachlorobenzene from the intestinal tract appears to depend on the solvent vehicle used during test material administration. Thus, when hexachlorobenzene is administered in olive oil, ~80% of the dose is absorbed; when it is administered in an aqueous solution, in 1% methyl cellulose or in a crystalline form, relatively little (<20%) is absorbed. Intestinal absorption of hexachlorobenzene occurs primarily through lymphatic channels, with only a minor portion being absorbed into the portal circulation.

Following absorption, hexachlorobenzene distributes to tissues that have a high lipid content. The adipose tissue accumulates the greatest concentrations of hexachlorobenzene in all species studied, although bone marrow and skin, which contain large amounts of lipids, also accumulate hexachlorobenzene. The adrenal cortex accumulates hexachlorobenzene at concentrations approaching those of fat. Other tissues (e.g., liver, kidneys, lungs, heart, spleen and blood) generally contain much lower amounts of hexachlorobenzene. Intravenous injection of hexachlorobenzene results in a tissue distribution similar to that seen following oral administration. Hexachlorobenzene is transported via the placenta and is distributed in fetal tissue in rabbits, rats, mice, minks and ferrets.

Hexachlorobenzene is metabolized slowly into other chlorinated benzenes, chlorinated phenols and other minor metabolites and forms glucuronide and glutathione conjugates. Tissues were found to contain mainly unchanged hexachlorobenzene together with small amounts of metabolites. Similarly, only small amounts of hexachlorobenzene metabolites were detected in feces, whereas most of the metabolites were excreted in the urine together with small amounts of unchanged hexachlorobenzene. There are indications that females produce and excrete more hexachlorobenzene metabolites than do males.

The excretion of hexachlorobenzene from treated animals is slow and occurs mainly through the feces with relatively little being excreted in the urine. It is characterized by an initial rapid phase followed by one or

more slow phases. This slow phase of excretion can be enhanced by the administration of mineral oil, paraffin or n-hexadecane. Both biliary and intestinal excretion contribute to fecal excretion. A three-compartment mammillary model has been reported for the behavior of hexachlorobenzene in beagles and rhesus monkeys following i.v. injection of a single dose. Radioactivity was not detected in exhaled air following i.p. injection of ^{14}C-hexachlorobenzene. Hexachlorobenzene has been detected in the milk of nursing mammals.

IV. HUMAN EXPOSURE

Humans may be exposed to chemicals such as hexachlorobenzene (HCB) from a variety of sources, including drinking water, food, ambient air, occupational settings, and consumer products. This analysis of human exposure to HCB is limited to drinking water, food, and ambient air because those media are considered to be sources common to all individuals. Even in limiting the analysis to these three sources, it must be recognized that individual exposure will vary widely based on many personal choices and on several factors over which there is little control. Where one lives, works, and travels, what one eats, and physiologic characteristics related to age, sex, and health status can all profoundly affect daily exposure and intake. Individuals living in the same neighborhood or even in the same household can experience vastly different exposure patterns.

In the Exposure Estimation section of this chapter, available information is presented on the range of human exposure and intake for HCB from drinking water, food, and ambient air for the 70-kg reference man. It is not possible to provide an estimate of the number of individuals experiencing specific combined exposures from those three sources. However, the Summary section of this chapter provides some insight into the relative contributions of the three sources, especially drinking water, to the range of intake values suggested by the available data.

Exposure Estimation

a. Drinking Water

Very limited information is available on levels of hexachlorobenzene in finished drinking water supplies. Only one Federal survey and one regional survey include analyses for HCB.

The National Screening Program for Organics in Drinking Water (NSP) (Boland 1981), conducted by SRI International from June 1977 to March 1981, examined levels of styrene finished water samples from 114 water facilities in 33 states. Samples were analyzed for many halocarbons, pesticides, and other chemicals (including HCB). HCB was assayed in finished water from 12 groundwater supplies and 104 surface water supplies. The compound was not detected at a quantification limit of 0.1 ug/l.

In a Region V study by the U.S. Environmental Protection Agency (EPA) (USEPA 1975), drinking water supplies from 83 locations were analyzed for various pesticides, PCB's, and other organic chemicals. HCB was detected finished water from in two supplies at levels of 0.004 and 0.006 ug/l. The source of the water, number of samples, and detection limit were not reported.

The levels of HCB observed in drinking water when it has been found are in the very low ug/l range. Assuming that HCB is present at the highest concentration reported, i.e., 0.006 ug/l in EPA Region V, the daily intake for the 70-kg adult male consuming 2 liters of drinking water per day would be 0.012 ug/day or 0.0002 ug/kg/day.

b. Diet

Limited information was obtained on dietary exposure to HCB. Dietary HCB intake has been reported for adults, as well as infants and toddlers, in the Food and Drug Administration's (FDA) Market Basket Studies for fiscal year 1979 (FDA 1982a, 1982b). Total dietary intake of HCB for adults in FY79 was 0.223 ug/day (FDA 1982a). [This included 0.093 ug/day from dairy products; 0.099 ug/day from meat, fish, and poultry; 0.005 ug/day from potatoes; and 0.027 ug/day from oils and fats.] Assuming that the average adult male weighs 70 kg, an intake of 0.223 ug/day would be equivalent to 0.0032 ug/kg/day. [Note: The FDA study reported dietary intake data for a 69.1 kg adult male. The calculations made for this section (as well as for air and drinking water) are based on a 70-kg adult male, which is used as a reference body weight by the Office of Drinking Water.] Given the lack of additional data, the dietary intake of HCB for the adult male was assumed to be at or near that level.

The relative daily intakes of HCB calculated by FDA for FY74 through FY79 are presented in Table IV-I. It is apparent from the table that the daily intake values calculated for these years fluctuate; no trend is observed for the intake levels.

Total dietary intakes of HCB for infants and toddlers in FY79 were 0.083 ug/day and 0.108 ug/day, respectively (FDA 1982b). These values included: 0.033 ug/day from whole milk, 0.010 ug/day from other dairy and substitutes, 0.005 ug/day from meat, fish, and poultry, and 0.035 ug/day from oils and fats in the infant study; and 0.031 ug/day from whole milk, 0.019 ug/day from other dairy and substitutes, 0.032 ug/day from meat, fish, and poultry, and 0.025 ug/day from oils and fats in the toddler study.

Table IV-I. Estimated Dietary Intake of HCB for Adults, Infants, and Toddlers (ug/kg/day)

	Adult	Infant	Toddler
FY79	0.0032	0.0101	0.0078
FY78	0.0039	0.0116	0.0145
FY77	0.0018	0.0382	0.0219
FY76	0.0019	0.0009	0.0042
FY75	0.0046	0.0044	0.0064
FY74	0.0010	—	—

Assumptions: 70-kg adult male, 8.2 kg infant, 13.7 kg toddler.
-- = Data not obtained.
Source: FDA, 1980a,b; 1982a,b

(Intake values may not add to the total due to rounding.) Assuming that the average infant (6 month old) weighs 8.2 kg and the average toddler (2 years old) weighs 13.7 kg, intakes of 0.083 ug/day for the infant and 0.108 ug/day for the toddler would be equivalent to 0.0101 ug/kg/day and 0.0078 ug/kg/day, respectively.

Calculations of intake for infants and toddlers made by FDA for FY75 through FY79 are presented in Table IV-I. The infant/toddler intake values appear to fluctuate over the years, and no particular trend is observed for the intake levels.

FDA compared estimated intakes of HCB for infants and toddlers by geographic region in FY77 (FDA 1980b). Intakes for both infants and toddlers were highest in the Northeastern Region. In FY77, the average infant intake was approximately 0.038 ug/kg/day, while the intake for the Northeastern region was 0.143 ug/kg/day. For toddlers, the average intake in FY77 was 0.22 ug/kg/day, and the estimate for the Northeastern region was 0.062 ug/kg/day.

c. Air

No information was available from the EPA Office of Air Quality Planning and Standards on the occurrence of HCB in ambient air. Consequently, respiratory intake and the contribution of respiratory intake to total exposure could not be evaluated.

Summary

Table IV-II presents a general view of the total amount of HCB received by a 70-kg adult male from air, food, and drinking water. Three separate exposure levels in food, four exposure levels in drinking water, and one exposure level from air are shown in the table.

The data presented have been selected from an infinite number of possible combinations of concentrations for the three sources. Whether exposure occurs at any specific combination of levels is not known; nor is it possible to determine the number of persons that would be exposed to benzene at any of the combined exposure levels. The data presented represent possible exposures based on the occurrence data and the estimated intakes.

There is no information available on the presence of HCB in ambient air; it is assumed here that air does not contribute to the daily intake of HCB.

The data for food presented in Table IV-II have been chosen as representative of potential adult dietary intake values based on values reported by FDA during the fiscal years 1974-1979 (see Table IV-I).

Intake values for drinking water, presented in Table IV-II, have also been chosen from a range of possible values. Although the highest level of HCB actually found in drinking water was 0.006 ug/l. (see a. Drinking Water), higher values have been included here to indicate the

Table IV-II. Estimated Intake of Hexachlorobenzene from the Environment by Adult Males in ug/kg/day

	Estimated total intake in ug/kg/day (% from drinking water) based on food levels of:		
Concentration in drinking water (ug/l)	Low (0.001 ug/kg/day)	Intermediate (0.0025 ug/kg/day)	High (0.005 ug/kg/day)
0.0	0.001 (0%)	0.0025 (0%)	0.005 (0%)
0.005	0.0011 (12%)	0.0026 (5%)	0.0051 (3%)
0.1	0.0039 (74%)	0.0054 (54%)	0.0079 (37%)
5.0	0.14 (99%)	0.14 (98%)	0.15 (97%)

Intake from each source:

Water: 0.005 ug/l: 0.00014 ug/kg/day
0.1 ug/l: 0.0029 ug/kg/day
5.0 ug/l: 0.14 ug/kg/day

Food: 0.001 ug/kg/day
0.0025 ug/kg/day
0.005 ug/kg/day

Air: 0.0 ug/m^3: 0.0 ug/kg/day

potential relative contribution of drinking water to total exposure that would occur if these higher levels actually were found in drinking water. It is assumed that the 70-kg adult male consumes 2 liters of drinking water per day.

The mid-range concentration of HCB used for food intake was 0.0025 ug/kg/day. Assuming this to be the contribution from food and the estimated HCB intake of 0.0 ug/m^3 from air, drinking water would be the predominant source of HCB exposure in the adult male at drinking water levels above 0.09 ug/l. An accurate assessment of the number of individuals for which drinking water is the predominant source of exposure cannot be determined from the data since specific locations containing high concentrations of HCB in drinking water and low concentrations of HCB in ambient air and food are unknown.

The relative source contribution data are based on estimated intake and do not account for a possible differential absorption rate for HCB by route of exposure. The relative dose received may vary from the relative intake. In addition, the relative effects of the chemical on the body may vary by different routes of exposure.

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V. HEALTH EFFECTS IN EXPERIMENTAL ANIMALS

Acute Toxicity

Information on the acute toxicity of hexachlorobenzene was limited to oral LD_{50} values determined with a few mammalian species. The following LD_{50} values were reported in the available literature: rats, 3500-10,000 mg/kg; rabbits, 2600 mg/kg; cats, 1700 mg/kg; and mice, 4000 mg/kg (IARC, 1979; NAS, 1977; Sax, 1979).

Graef et al. (1979) reported that hexachlorobenzene blocked the activity of rat hepatic 3-hydroxysteroid dehydrogenase leading to the accumulation of 5β-H-steroids, which are known inducers of porphyrin biosynthesis. Hexachlorobenzene-induced porphyria has also been reported to occur as a result of a deficiency in the uroporphyrinogen decarboxylation process that is catalyzed by porphyrinogen carboxylase. This enzyme is the only one in the heme pathway that exhibits a decrease in activity. The inhibition of porphyrinogen carboxylase in liver homogenates from female Wistar rats with severe porphyria induced by hexachlorobenzene was studied by Rios de Molina et al. (1980). Hexachlorobenzene had no effect on enzyme activity at 10^{-3} M, whereas pentachlorophenol caused a 90% inhibition at the same concentration. However, pentachlorophenol did not inhibit the enzyme at a concentration of 10^{-5} M. It was concluded that a concentration $>10^{-5}$ M of pentachlorophenol, possibly together with other hexachlorobenzene metabolites, was needed to cause enzyme inhibition.

Hexachlorobenzene has also been reported to induce the activity of hepatic microsomal enzymes in male or female rats following subchronic administration (Carlson, 1978; Carlson and Tardiff, 1976; Chadwick et al., 1977). Hexachlorobenzene produced a so-called "mixed-type" induction of cytochrome P-450 content in female rats resembling that produced by a combination of phenobarbital (cytochrome P-450) and 3,4-benzopyrene (cytochrome P-448) (Goldstein et al., 1982; Debets et al., 1980a). In female rats, hexachlorobenzene increased the activities of δ-aminolevulinic acid synthetase and aminopyrine demethylase (Ariyoshi et al., 1974), ethoxyresorufin-O-deethylase, aminopyrine demethylase, aryl hydrocarbon hydroxylase, p-nitrophenol glucuronyl transferase, and NADPH-cytochrome c reductase (Goldstein et al., 1982; Debets et al., 1980a). Similarly, in male rats, hexachlorobenzene increased the activities of hepatic ethyl morphine N- and p-nitroanisol O-demethylases, aniline hydroxylase, and UDP glucuronyl transferase (Mehendale et al., 1975), acetanilide hydroxylase, acetanilide esterase, procaine esterase, and arylesterase activities (Carlson et al., 1979; Carlson, 1980).

Subchronic Toxicity

Several oral subchronic studies of hexachlorobenzene have been reported, but no studies were located on the effects of hexachlorobenzene following inhalation. In several animal species, hexachlorobenzene was found to cause alopecia and scabbing, decreased body weight, increased liver and kidney weights and increased porphyrin levels in the urine and in several organs. Histopathologic changes were noted in the liver and kidneys of rats, gastric lymphoid tissue of dogs, and ovaries of monkeys. When placed on untreated diets, rats were able to recover from most of the toxic effects of hexachlorobenzene treatment. Hexachlorobenzene was also reported to cause certain neurologic effects (ataxia, paralysis, etc.) on rats, mice, hamsters and female beagles, and to induce an increase in hepatic microsomal enzyme activity. Toxicity data for hexachlorobenzene can be found in Table V-1.

TABLE V-1

Summary of Toxicity Studies on Hexachlorobenzene

Species	Route	Dose	Duration	Effects	Reference
Rat (females)	oral	100 mg/kg every other day	up to 43 days	Suggested covalent binding of hexachlorobenzene metabolites to cytosolic proteins	Koss et al., 1980a
Rat	oral (diet)	0.5 mg/kg/day	15 weeks exposed and held to 48 weeks	Transient increases in liver porphyrin levels in females after termination of exposure	Kuiper-Goodman et al., 1977
		2.0 mg/kg/day	15 weeks exposed and held to 48 weeks	Increases in liver porphyrin levels in females after termination of exposure, increased size of centrilobular hepatocytes	
		8.0 mg/kg/day	15 weeks exposed and held to 48 weeks	Increased liver weights, increased liver, kidney and spleen porphyrin levels in females (porphyria), centrilobular liver lesions especially in females at 48 weeks	
		32.0 mg/kg/day	15 weeks exposed and held to 48 weeks	Increased mortality in females, intension tremors in males and females and ataxia in a few females, increased liver, kidney and spleen weights, increased liver, kidney and spleen porphyrin levels in females (porphyria), centrilobular liver lesions and splenomegaly	
Rat (females)	oral (gavage)	50 mg/kg every other day	15 weeks	Increased liver, kidney, spleen and adrenal weights, porphyria (increased liver porphyrin levels and increased excretion of porphyrins and precursors), tremors, hair loss and skin lesions	Koss et al., 1978b
Rats (females)	oral (gavage)	0.5 mg/kg twice weekly	29 weeks	Increase in relative liver weight	Böger et al., 1979
		2.0 mg/kg twice weekly	29 weeks	Increase in relative liver weight, moderately enlarged hepatocytes	
		8.0 mg/kg twice weekly	29 weeks	Porphyria, markedly enlarged hepatocytes, increase in relative liver weight	
		32.0 mg/kg twice weekly	29 weeks	Porphyria, markedly enlarged hepatocytes, increase in liver weights	
Rat (females)	oral (diet)	100 mg/kg diet	90 days	Porphyria (increased liver lobe porphyrins), decreased activity of uroporphyrinogen decarboxylase	Smith et al., 1980

TABLE V-1 (cont.)

Species	Route	Dose	Duration	Effects	Reference
Rat	oral (diet and nursing)	50 mg/kg diet	gestation until 5 weeks of age	Depressed resistance to L. monocytogenes and T. spiralis, enhanced thymus-dependent antibody response	Vos et al., 1979b
		150 mg/kg diet	gestation until 5 weeks of age	Increased serum IgM and IgG, depressed resistance to L. monocytogenes and T. spiralis, enhanced thymus-dependent antibody response, increased liver and adrenal weights	
Rat	oral (diet)	500, 1000 or 2000 mg/kg diet	3 weeks	Dose-related increases in relative spleen, lymph nodes, liver, adrenals, thyroid, testes and kidney weights, dose-related increase in serum IgM levels, no change in serum IgG levels, dose-related pathological changes in liver, lymph nodes and spleen	Vos et al., 1979a
Rat	oral (diet)	2000 mg/kg diet	10 weeks	Porphyria found microscopically at 5 weeks and grossly at 10 weeks using fluorescence	Gralla et al., 1977
Rat (male)	oral (diet)	2000 mg/kg diet	100 days	Elevated hepatic enzymes by 1 week and increased urinary porphyrin and ALA levels (porphyria) as early as 40 days	Lissner et al., 1975
Rat (female)	oral (diet)	3000 mg/kg diet	11 weeks	Decreased uroporphyrinogen decarboxylase activity and porphyria after 4 weeks	Elder et al., 1976
Rat (female)	oral (gavage)	50, 100 or 200 mg/kg	120 days	Dose- and time-dependent increase in liver and urine porphyrins (porphyria)	Carlson, 1977
Rat	oral (gavage)	14 mg/kg every other day	103 days	Porphyria in treated females, susceptibility of females to porphyria may be related to estrogen levels	Rizzardini and Smith, 1982
Rat (females)	oral (gavage)	100 mg/kg every other day	6 weeks exposed and held for additional 18 months	Porphyria (liver uroporphyrin levels peaked 7 months postexposure and levels had not returned to normal by 18 months), decreased liver protoporphyrin and coproporphyrin levels, inhibition of uroporphyrinogen decarboxylase activity until 18 months postexposure	Koss et al., 1983
Rat (females)	oral (diet)	6-8 mg/kg/day	75-90 weeks	Decline in body weights, porphyria, enlarged livers and liver tumors	Smith and Cabral, 1980
Rat	oral (diet)	75 mg/kg diet (4-5 mg/kg/day) 150 mg/kg diet (8-9.5 mg/kg/day)	up to 2 years	Porphyria, time-related appearance of severe hepatic and renal pathologies, after 1 year increases in hepatomas, hepatocarcinomas, bile duct adenomas, renal adenomas and renal carcinomas	Lambrecht et al., 1983a,b

TABLE V-1 (cont.)

Species	Route	Dose	Duration	Effects	Reference
Rat	oral (diet)	0.32, 1.6, 8.0 or 40 mg/kg diet	~130 days	Hematological changes at all dose levels in males, increases in liver and heart weights in males at 8.0 and 40 ppm diets, no treatment-related effects observed in bred females	Arnold et al., 1985
	oral (diet and nursing)	0.32 or 1.6 mg/kg diet	gestation through lifetime (130 weeks)	Glycogen depletion in 1.6 mg/kg males; no effects reported at 0.32 mg/kg	
		8.0 mg/kg diet	gestation through lifetime (130 weeks)	Increase in liver pathologies	
		40 mg/kg diet	gestation through lifetime (130 weeks)	Increased mortality as pups, increase in liver and kidney pathologies, increase in adrenal pheochromocytomas in females and parathyroid tumors in males	
Rat	oral (diet)	10 or 20 mg/kg diet	F_0 to F_4 generations	No effects reported	Grant et al., 1977
		40 mg/kg diet	F_0 to F_4 generations	Increases in liver weights and aniline hydroxylase activity	
		80 mg/kg diet	F_0 to F_4 generations	Decreased body weights, F_3 and F_4 generations had decreased lactation index and postnatal viability and increased stillbirths	
		160 mg/kg diet	F_0 to F_4 generations	Increased mortality and decreased lactation index starting in F_1 generation	
		320 and 640 mg/kg diet	F_0 to F_4 generations	20 and 50% mortality in F_0 320 and 640 mg/kg groups, respectively, greatly reduced fertility index and litter size and increase in stillbirths, viability index zero in F_1	
Rat	oral (diet)	60, 80, 100, 120 or 140 mg/kg diet	F_0 to F_{1a} and F_{1b} generations	Increased mortality in all groups at 21 days, 21-day LD_{50} values for pups were 100 and 140 mg/kg for F_{1a} and F_{1b} generations, respectively	Kitchin et al., 1982
Rat	oral (diet)	0 or 80 mg/kg diet	gestation and nursing or cross nursed with controls	Nursing exposure produced greater effects than did gestational exposure, effects noted were: smaller brains, hearts, kidneys and spleens, increased liver weights	Mendoza et al., 1978
Rat	oral (diet)	80 mg/kg diet	2 weeks prior to mating to 35-36 days after weaning	Increased porphyrin levels and decreased liver esterase activity in dams, no changes in gestation indices or neonatal survival	Mendoza et al., 1979

TABLE V-1 (cont.)

Species	Route	Dose	Duration	Effects	Reference
Rat	oral (gavage)	10, 20, 40, 60, 80 or 120 mg/kg	days 6-21 of gestation	Maternal toxicity (weight loss, tremors and convulsions) and reduced fetal weights at 120 and 80 mg/kg maternal doses, dose-related increase in incidence of unilateral and bilateral 14th rib, sternal defects were also noted in one experiment	Khera, 1974
Mouse	oral (diet)	2.5, 25 or 250 mg/kg diet	21 days	Dose-related increase in liver and decrease in prostate and seminal vesicle weights, dose-related alterations in testosterone metabolism, altered hepatic enzyme levels	Elissalde and Clark, 1979
Mouse (male)	oral (diet)	10 mg/kg diet (8.4 (mg/mouse/24 weeks) or 50 mg/kg diet (35.3 mg/mouse/ 24 weeks)	24 weeks	Dose-related reduction in weight gain, no tumor pathology observed	Shirai et al., 1978
Mouse (male)	oral (diet)	167 mg/kg diet	3-6 weeks	Impairment in host resistance as measured by increased sensitivity to *S. typhosa* and *P. bergheri*, and decrease in IgA levels	Loose et al., 1978a,b
Mouse	oral (diet)	6, 12, 24 and 36* mg/kg/day	101-120 weeks *(15 weeks exposed held until 120 weeks)	Reduced growth rate at all dose levels, shortened lifespan associated with tremors and convulsions in 24 and 36 mg/kg/day groups, dose-dependent increase in liver-cell tumors in the 12, 24 and 36 mg/kg/day dose groups	Cabral et al., 1979
Mouse	oral (gavage)	100 mg/kg/day to pregnant mice	days 7-16 of gestation	Increased maternal livers and decreased fetal body weights, increased incidence of abnormal fetuses per litter observed	Courtney et al., 1976
Hamster	oral (diet)	200 or 400 mg/kg diet	90 days	Precirrhotic and cirrhotic hepatic lesions, bile-duct hyperplasias and hepatomas	Lambrecht et al., 1982
Hamster	oral (diet)	4, 8 or 16 mg/kg/day	lifespan	Shortened lifespan in 16 mg/kg/day group, increase in hepatomas at all dose levels, increase in liver haemangioendothelioma in males and females and an increase in thyroid alveolar adenomas in males in 16 mg/kg/day group	Cabral et al., 1977
Cats (breeding females)	oral (diet)	3 or 8.7 mg/day/cat	142 days	Weight loss and increased disease susceptibility in bred females, dose-related decrease in litter size and survival of offspring, hepatomegaly in offspring	Hansen et al., 1979

TABLE V-1 (cont.)

Species	Route	Dose	Duration	Effects	Reference
Minks	oral (diet)	1 or 5 mg/kg diet	during gestation until 17 weeks of age	Dose-related increase in offspring mortality, induction of hepatic MFO enzymes in exposed offspring	Rush et al., 1983
Dog (female)	oral (capsule)	50 or 150 mg/kg/day	21 days	Liver and hepatocyte enlargement, dose-induced electroencephalogram dysrhythmias	Sundlof et al., 1981
Dog	oral (capsule)	1, 10, 100 or 1000 mg/day/dog	1 year	Increase in mortality, neutrophilia, and anorexia in the 100 and 1000 mg dose groups, dose-related nodular hyperplasia of gastric lymphoid tissue in all treated animals	Gralla et al., 1977
Monkey (female)	oral (gavage)	8, 32, 64 or 128 mg/kg/day	60 days	Dose-related pathology in liver, kidney, ovaries and thymus	Iatropoulus et al., 1976
Monkey	oral (nursing)	7.51-186 ppm milk	60 days	2 of 3 infants died as a result of exposures	Bailey et al., 1980

Iatropoulos et al. (1976) reported that five adult female rhesus monkeys given daily gavage treatments of hexachlorobenzene suspended in 1% carboxymethylcellulose at 8, 32, 64 or 128 mg/kg/day for 60 days, showed extensive morphologic changes in the ovaries. These changes were dose-related.

Subchronic studies conducted by Koss et al. (1980a) with groups of four female rats treated orally (probably by gavage) with 100 mg/kg of hexachlorobenzene in olive oil every other day, suggested that hexachlorobenzene metabolites covalently bind to cytosolic proteins although no binding to uroporphyrinogen decarboxylase was specifically demonstrated.

Elissalde and Clark (1979) reported a significant increase in the _in vitro_ metabolism of ^{3}H-testosterone by liver microsomes from male mice fed diets containing 250 mg hexachlorobenzene/kg for 21 days. In addition, decreases in the concentration of testosterone in the serum and in the weights of seminal vesicles and ventral prostates were reported. Hexachlorobenzene was also reported to cause certain neurologic lesions in male and female rats, hamsters and mice fed diets containing varous levels of hexachlorobenzene for 13 weeks. These included hyperemia, edema, arborization and hemorrhages in the brain and meninges. The lesions extended to the cerebrum, cerebellum, medulla, spinal cord and their meninges. The severity of these lesions was higher in males and was dose dependent in both sexes (Headley et al., 1981). Physiologic changes (electroencephalogram dysrhythmias) in the central nervous system were reported in 10 female beagles receiving gelatin capsules containing doses of 50 or 150 mg/kg of hexachlorobenzene for 21 days (Sundlof et al., 1981).

Kuiper-Goodman et al. (1977) conducted a 15-week subchronic feeding study wherein groups of 70 male and 70 female COBS rats were fed diets providing 0, 0.5, 2, 8 or 32 mg/kg bw/day of hexachlorobenzene originally dissolved in corn oil (5%) and mixed with the feed. Female rats were more susceptible to hexachlorobenzene than males, as indicated by all the parameters studied, and a NOEL of 0.5 mg/kg/ day was suggested by the authors. This NOEL may be better interpreted as a NOAEL since a transient increase in liver porphyrin levels was observed in females 4 weeks after removal from hexachlorobenzene. The 2 mg/kg/day dose may be interpreted as a LOAEL since this level caused increases in liver porphyrin levels in females even 33 weeks after removal from hexachlorobenzene, and increases in the relative observed severity of centrilobular liver lesions as compared to control rats. About 40% mortality occurred in females, but none in males at the highest dose. Clinical signs included intention tremor, excessive irritability, multiple alopecia, scabbing and ataxia, with hind leg paralysis at the highest dose. There was a significant increase in liver and kidney weights at the higher doses. An increase in liver weight was also found in groups of 36 female Wistar rats treated by gavage twice weekly with hexachlorobenzene dissolved in olive oil at 32 mg/kg for 29 weeks (Boger et al., 1979). Similarly, Koss et al. (1978b) reported a 1.5- to 2-fold increase in the weights of the liver, spleen, kidneys and adrenal glands from female Wistar rats treated orally (esophageal tube) with 50 mg/kg of hexachlorobenzene dissolved in corn oil every other day for 15 weeks. When hexachlorobenzene-treated rats were placed on untreated diets, they no longer showed signs of hexachlorobenzene toxicity, such as dermal lesions, and body and organ weights returned to normal (Kuiper-Goodman et al., 1977; Koss et al., 1978b). Enlarged livers were reported in subchronic studies with female beagles (Sundlof et al., 1981) and male mice (Shirai et al., 1978) administered hexachlorobenzene in diet.

A dose-dependent enlargement of hepatocytes was observed in groups of 36 female Wistar rats receiving gavage treatments of olive oil containing hexachlorobenzene (99.8% pure) 0.5, 2.0, 8.0 and 32 mg/kg twice weekly for 29 weeks (Boger et al., 1979). This effect was associated with the proliferation of the smooth endoplasmic reticulum in the centrilobular area, and an increase in glycogen deposits; however, animals receiving 0.5 mg/kg did not develop enlarged hepatocytes. In addition, atypical membrane complexes in treated animals were noted and liver-cell mitochondria were moderately enlarged and had irregular shapes. Kuiper-Goodman et al. (1976) also reported significantly enlarged hepatocytes in male and female COBS rats receiving hexachlorobenzene in diets, containing 5% corn oil, at the 8.0 and 32.0 mg/kg bw dose levels for 15 weeks. They observed that this hepatocyte enlargement consisted to a large degree of proliferation of the smooth endoplasmic reticulum. In males this proliferation was often associated with large whorls of compacted membranes surrounding lipid droplets. The nuclei of enlarged hepatocytes were also enlarged while the mitochondria were very small and sparse. They stated that this proliferation of smooth endoplasmic reticulum was related to the increased drug metabolizing enzyme activity of the liver and was considered an adaptive rather than toxic response to the hexachlorobenzene, since the enzyme activity and liver morphology returned to normal after exposures were discontinued. An increase in the size of centrilobular hepatocytes was also reported in male and female rats receiving 2 mg/kg/day for 15 weeks, together with histopathologic changes in the spleen (Kuiper-Goodman et al., 1977).

Nodular hyperplasia of gastric lymphoid tissue was reported in groups of 6 male and 6 female beagles receiving daily gelatine capsules containing 1, 10, 100 and 1000 mg hexachlorobenzene/dog/day for 12 months (Gralla et al., 1977). Extensive dose-related histopathologic changes were also observed in ovaries from groups of two rhesus monkeys given daily methyl cellulose/ distilled water solutions containing doses of 8, 16, 32, 64 or 128 mg hexachlorobenzene/kg of body weight by gavage for 60 days (Knauf and Hobson, 1979; Iatropoulas et al., 1976). Shirai et al. (1978) conducted a 24-week study with male mice fed diets containing 10 or 50 ppm of hexachlorobenzene, followed by a recovery period of 14 weeks. Histologic examination revealed no pathologic changes in the liver or any other organ.

Lambrecht et al. (1982) fed male and female Syrian golden hamsters hexachlorobenzene at doses of 0, 200 and 400 ppm in their diet for 90 days. The hamsters were killed on day 91 and at 6-week intervals through day 361. No differences were seen in growth and food consumption between control and exposed animals. The liver was reported as the most severely affected organ exhibiting a variety of precirrhotic and cirrhotic lesions, bile-duct hyperplasias and hepatomas. The incidence of neoplasms found in this study will be further discussed in the Carcinogenicity Section.

Hexachlorobenzene has been found to cause increased porphyrin levels in the liver of male and female rats receiving the compound incorporated into the diet at doses of 8 and 32 mg/kg/day for 15 weeks (Kuiper-Goodman et al., 1977). Koss et al. (1978b) reported that female rats treated orally with 50 mg hexachlorobenzene/kg every other day for 15 weeks still showed increased levels of porphyrin in the liver, 38 weeks after the last treatment. In addition, porphyrin, δ-aminolevulinic acid, and porphobilinogen levels in the urine gradually increased during the 15-week treatment period, but subsequently decreased to normal levels. Smith et al. (1980) reported that the lobes of livers from female Agus rats fed diets containing 0.01% hexachlorobenzene developed porphyria at different rates. During the initial course of treatment, porphyria in the caudate lobe developed at a significantly slower rate than the median, left or right sections of the liver, but eventually, all lobes became equally porphyric. In contrast, porphyria was not observed when viewed for hepatic fluorescence of porphyrins in male and female beagle dogs treated daily with 0, 1, 10, 100 or 1000 mg/dog/day for 1 year (Gralla et al., 1977). Gralla et al. (1977) observed that female CD rats fed 0.2% hexachlorobenzene were porphyric using this fluorescence method.

Rizzardini and Smith (1982) clearly confirmed that female rats are more susceptible to hexachlorobenzene-induced porphyria than are male rats, and that this difference in susceptibility is probably associated with the faster metabolism of hexachlorobenzene in females. They intubated male and female F344/N rats every other day for 103 days with 14 mg/kg (50 μmole/kg) hexachlorobenzene dissolved in arachis oil and monitored the rats for hexachlorobenzene metabolites and porphyrin levels. The results indicated that after 75 days of hexachlorobenzene treatment the excretion of urinary porphyrins increased rapidly in the females and after 103 days the females had urine and liver porphyrin levels 40- and 310-fold higher, respectively, than did the males. During this treatment period the females were found to excrete greater quantities of hexachlorobenzene metabolites, especially pentachlorothiophenol, than the males. Estrogen levels seem to play an important part in the increased susceptibility of females to hexachlorobenzene-induced porphyria. When both male and female rats were pretreated intraperitoneally with four doses of 20 μmole/kg of diethylstilboestrol dipropionate (an estrogenic drug), both sexes had stimulated excretion of hexachlorobenzene metabolites.

Hexachlorobenzene pretreatment has been reported to cause altered immune responses. Vos et al. (1979b) studied the effect of hexachlorobenzene on the immune system after combined pre- and postnatal exposure. Wistar rats were fed diets containing 50 or 150 µg/kg hexachlorobenzene during pregnancy and lactation. The pups were weaned after 3 weeks and continued on the test diets until 5 weeks of age, when their immune system was functionally assessed. At the higher dietary level, hexachlorobenzene caused a statistically signficant increase in serum IgM and IgG concentrations.

Hexachlorobenzene treatment also caused a decreased resistance to infection with *Listeria monocytogenes* (Vos et al., 1979b). The LD_{50} values were reported to be 14x10^{5}, 7.1x10^{5} and 5.0x10^{5} bacteria in pregnant Wistar rats receiving diets containing 0, 50 and 150 mg/kg, respectively. Similarly, decreased resistance of *Trichinella spiralis* infection, as indicated by an increase in the number of larvae found in muscle tissue, was noted. Hexachlorobenzene also enhanced the thymus-dependent antibody response to *T. spiralis* antigen and to tetanus toxoid. No effects were observed on allograft rejection, mitogenic response of thymus and spleen cells, thymus-independent IgM response to *Escherichia coli* lipopolysaccharide, passive cutaneous anaphylaxis, and on the clearance of carbon particles and *L. monocytogenes*. The authors concluded that hexachlorobenzene suppressed cellular immunity and enhanced humoral immunity in both test groups.

In contrast, hexachlorobenzene pretreatment of weanling rats did not alter their cell-mediated immunity, but did stimulate their humoral immune response and enhanced the *in vitro* responsiveness of spleen cells to different mitogens, which was mainly a result of an increase in the number of splenic lymphocytes. The rats received diets containing 1000 μg hexachlorobenzene/g for 3 weeks after weaning, before assessing their immune system (Vos et al., 1979a).

Loose et al. (1978a,b) found that hexachlorobenzene pretreatment also resulted in impaired host resistance. Male BALB/c mice received diets containing 167 μg hexachlorobenzene/g for 3 or 6 weeks before assessing their immune functions. The concentration of IgA was significantly decreased, whereas those of IgG and IgM did not exhibit consistent significant alterations as compared with the controls. Hexachlorobenzene-treated mice were more sensitive to gram-negative endotoxin (Salmonella typhosa), showed a decreased resistance to a malaria challenge (Plasmodium berghei), and exhibited signficantly depressed antibody synthesis.

Chronic Toxicity

Cabral et al. (1977) studied the tumorigenicity of hexachlorobenzene in 6-week-old Syrian golden hamsters given 0, 50 (4 mg/kg/day), 100 (8 mg/kg/day) and 200 (16 mg/kg/day) ppm hexachlorobenzene in their diets for their remaining lifespan. Shortened lifespan was observed in the male and female 200 ppm dose groups after 70 weeks of exposure along with marked weight reduction in the males. Neoplasms were increased by the hexachlorobenzene exposures and are reported in the Carcinogenicity Section. No other pathologies were reported in this study.

Cabral et al. (1979) studied the tumorigenicity of 6- to 7-week-old male and female outbred Swiss mice given 0, 50 (6 mg/kg/day) 100 (12 mg/kg/day) and 200 (24 mg/kg/day) ppm hexachlorobenzene for 101-120 weeks and 300 ppm (36 mg/kg/day) hexachlorobenzene for 15 weeks and held until 120 weeks of age. Results indicated that shortened lifespan occurred in the 200 and 300 ppm dose groups starting after the 30th week of the test and that this reduced survival was associated with tremors and convulsions. Reduction in the rate of growth was observed in female mice in the 50, 200 and 300 ppm dose groups and more pronounced growth rate reduction was observed in male mice in the 100, 200 and 300 ppm dose groups. An increase in neoplasms were found as a result of hexachlorobenzene exposures and are discussed in the Carcinogenicity Section. No other pathologies were reported in this study.

Smith and Cabral (1980) fed young female Agus or MRC Wistar rats 100 ppm (6-8 mg/kg/day) hexachlorobenzene in a diet containing 2% arachis oil for 90 weeks. Hexachlorobenzene exposure resulted in a steady decline in body weights over the study period and in the exposed rats possessing less hair than the controls. Tremors or other nervous symptoms were not seen during this study. Onset of porphyria was observed in the hexachlorobenzene rats after ~3 months, as indicated by urines fluorescing red under UV light, and liver porphyria was confirmed at autopsy by a red fluorescence of the liver. The livers were enlarged 2-fold in the hexachlorobenzene-exposed females and were associated with multiple liver cell tumors. This neoplastic incidence will be discussed in the Carcinogenicity Section.

Male and female Sprague-Dawley rats were fed hexachlorobenzene diets for 2 years containing 0, 75 or 150 ppm hexachlorobenzene (Lambrecht et al., 1983a,b). Four rats per group were killed at weeks 0, 1, 2, 3, 4, 8, 16, 32, 48 and 64 of the study and liver and kidney evaluations were made. Times of appearance of lesions were as follows: 4 weeks -- hepatic hyperemia, edema, parenchymal and hydropic degeneration, renal hyperemia, congestion, swelling and parenchymal degenerations; 32 weeks -- renal tubular nephritis with hyaline casts, severe parenchymal degeneration, epithelial necrosis accompanied by proximal convoluted tubular regeneration, and preneoplastic foci; and 36 weeks -- hepatic preneoplastic foci; and 64 weeks -- hepatic neoplasms and renal neoplasms. The incidence of neoplasms will be further discussed in the Carcinogenicity Section.

A two-generation hexachlorobenzene (analytical grade) feeding study was conducted using Sprague-Dawley rats fed diets containing 0 (64 males, 64 females), 0.32 (40 males, 40 females), 1.6 (40 males, 40 females), 8.0 (40 males, 40 females), or 40.0 (66 males, 66 females) ppm hexachlorobenzene (Arnold et al., 1985). The parental rats (F_0) received their respective test diets for 90 days before mating and until 21 days after parturition (at weaning), at which time they were killed and evaluated for hexachlorobenzene-induced effects. The number of offspring (F_1 generation) from these matings were reduced to 50 males and 50 females per dose group at 28 days of age and fed their respective parents' diets. Thus, the F_1 animals were exposed to hexachlorobenzene and metabolites in utero, from maternal nursing and from their diets for the remainder of their lifetime (130 weeks).

The results from this two-generation study indicated no consistent treatment-related effects upon growth or food consumption in either generation and no change in fertility, gestation or lactation indices. A decreased viability index was noted in the 40.0 ppm group relative to controls. No treatment-related effects were found in the F_0 females. The F_0 males were found to have significantly increased liver, heart and brain absolute weights in the 8.0 ppm group and significantly increased liver and heart absolute weights in the 40.0 ppm group. The F_0 males were observed to have various significant changes in hematological parameters at all dose levels. Neoplasms were seen in the F_1 generation and are discussed in the Carcinogenicity Section. In the F_1 generation the following changes were seen:

1) Centrilobular basophilic chromogenesis showed a significant dose-related trend in both males and females. Additionally, at doses of 8.0 and 40.0 ppm the increases were significant in comparison with controls for both males and females.

2) Increases in peribilary lymphocytosis were statistically significant in the 0.32, 1.6 and 40.0 ppm male groups, while increases in peribiliary fibrosis were statistically significant in the 0.32 and 40.0 ppm male groups.

3) Increases in severe chronic nephrosis were observed which were dose-related, but statistically significant relative to controls only for the 40.0 ppm male dose group.

In a second study conducted by Arnold et al. (1985), 50 male Sprague-Dawley rats per group were fed hexachlorobenzene (0 or 40 ppm) and various levels of vitamin A diet (0.1, 1 or 10 times normal control levels). The test groups were as follows: control diet; control diet plus 40 ppm hexachlorobenzene; 1/10 vitamin A diet; 1/10 vitamin A diet plus 40 ppm hexachlorobenzene, 10 times control vitamin A diet; and 10 times vitamin A diet plus 40 ppm hexachlorobenzene. Five rats per group were killed and evaluated both at 25 and 49 weeks and the remaining animals were killed and evaluated after 119 weeks.

Results revealed that the animals on the 1/10 vitamin A diet had significantly reduced body weights and survivability when compared with control diet animals. The animals on 1/10 vitamin A plus 40 ppm hexachlorobenzene diet had significantly decreased body weights and did not survive as long as rats receiving the control diet plus 40 ppm hexachlorobenzene. Hematological evaluations revealed no consistent treatment-related effects. Neoplasms were observed in the test animals and are discussed in the Carcinogenicity Section. No significant differences were found in the incidence of any pathological lesions between the test groups.

Mutagenicity

In a dominant lethal mutation study (Simon et al., 1979), male rats (strain not given) received by oral gavage 0, 70 or 221 mg hexachlorobenzene/kg body weight dissolved in corn oil for 5 consecutive days. A dose-dependent reduction in male reproductive performance was observed, but hexachlorobenzene did not induce dominant lethal mutations. Khera (1974) also reported a lack of dominant lethal mutations in Wistar rats following oral administration of 0, 20, 40 or 60 mg hexachlorobenzene/kg in 0.25% aqueous gum traqacanth for 10 consecutive days. In 14 sequential mating trials, no significant differences in the incidence of pregnancies, corpora lutea, live implants and deciduomas between the treated and control groups were observed. Mutagenic activity has been observed in a yeast, Saccharomyces cerevisiae, assay (Guerzoni et al., 1976). The mutagenicity of hexachlorobenzene was investigated in three strains of S. cerevisiae using reversion from histidine and methionine auxotrophy, and hexachlorobenzene was reported to be mutagenic at a minimum concentration of 100 ppm.

Lawlor et al. (1979) measured the activity of hexachlorobenzene in the Ames assay, strains TA98, TA100, TA1535, TA1537 and TA1538, at five unspecified dose levels both with and without metabolic acitivation by Aroclor 1254 induced rat liver microsomes. Hexachlorobenzene possessed no detectable levels of mutagenic activity in any of the Salmonella strains used either with or without microsomal activation. These results were reported in an abstract with few experimental details. In addition, this result is not unexpected because the Salmonella test system is generally insensitive to chlorinated compounds (Rinkus and Legator, 1980).

Carcinogenicity

Studies on the carcinogenic potential of hexa- chlorobenzene have been carried out on hamsters, mice and rats.

Hamster Studies.

Cabral et al. (1977) -- In one study on Syrian golden hamsters (Cabral et al., 1977) hexachlorobenzene was administered in the diet at 50, 100 or 200 ppm. These concentrations correspond to dosages of 4, 8 and 16 mg/kg/day based on body weight and food intake averages. The hexachlorobenzene was prepared by dissolution in corn oil which was then mixed with the feed. The feed was analyzed periodically to insure that the intended level of hexachlorobenzene was maintained (Mollner, 1983). The hexachlorobenzene preparation used in this study was 99.5% pure. Impurities reported to be present in some hexachlorobenzene preparations include chlorinated dibenzofuran and chlorinated dibenzo-p-dioxin, both members of classes of compounds which are carcinogens (Villeneuve et al., 1974). The dosages selected for this study were chosen in order to be comparable to those believed to be consumed by victims of accidental hexachlorobenzene ingestion in Turkey.

In this study on hamsters it was difficult to determine from the published report whether an MTD was reached or exceeded because the information on mortality and weight changes was not detailed enough for an unambiguous evaluation. Although mortality was monitored, the investigators only stated that 71% of the treated animals were alive at 50 weeks and that at the highest dose, 16 mg/kg bw/day, there was a reduced lifespan among treated animals after 70 weeks. The study was run for the lifetime of the animals, but the actual duration in weeks was not given. Since the investigators also reported "marked weight reduction" in the highest dose group one could conclude that the MTD may have been reached. However, in the absence of weight data definite conclusions cannot be made.

The tumor incidence among the hamsters is given in Table V-2. The incidence of hepatomas in males and females was statistically significant in all treated groups. The incidence of liver haemangioendothelioma in males and females was statistically significant in the high dose groups and in males in the middle dose groups. There was a significant dose-related trend for both tumor types. Three instances of metastases were found among the animals with liver haemangioendothelioma. No hepatoma metastases were found. One of the hepatomas in a female animal was found at necropsy at 18 weeks; the investigators did not indicate which dosage level this animal received.

Hamsters in the control groups showed no thyroid tumors but thyroid alveolar adenomas were significantly increased in the high dose males and there was a significant dose-related trend. In female thyroid tumors occurred in all treated groups of females but were not statistically significant.

Chemical induction of thyroid tumors has not been identified with chemically related compounds except for toxaphene, which is a mixture of chlorinated camphene derivatives. Other chemicals associated with induction of thyroid tumors are thioureas, thiouracils, 3-amino-4-ethoxyacetanilide, amitrok, o-anisidine, 2,4-diaminanisole sulfate, ethionamide, 4,4'-methylene

TABLE V-2

Tumor Incidence in Hamsters Given HCB in the Diet*

Group	Effective No.	TBA No.	%	No. of Tumors		More Than One Tumor		Thyroid		Hepatoma		Haemangioendotheliomas				Other	
												Liver		Spleen			
				No.	per Hamster	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Control	39 F	5	12.8	5	0.13	0	0	0	0	0	0	0	0	1	2.5	4	10.2
	40 M	3	7.5	3	0.08	0	0	0	0	0	0	0	0	0	0	3	7.5
50 ppm (4 mg/kg)	30 F	16	53.3	21	0.70	4	13.3	2	6.6	14	46.6	0	0	0	0	5	16.6
	30 M	18	60.0	27	0.90	8	26.6	0	0	14	46.6	1	3.3	1	3.3	11	36.6
100 ppm (8 mg/kg)	30 F	18	60.0	32	1.06	11	36.6	1	3.3	17	56.6	2	6.6	3	10.0	9	30.0
	30 M	27	90.0	45	1.50	14	46.6	1	3.3	26	86.6	6	20.0	3	10.0	9	30.0
200 ppm (16 mg/kg)	60 F	52	86.6	73	1.21	15	25.0	3	5.0	51	85.0	7	11.6	4	6.6	8	13.3
	57 M	56	98.2	87	1.52	27	47.3	8	14.0	49	85.9	20	35.0	4	7.0	6	10.5

*Source: Cabral et al., 1977

TBA = Tumor-bearing animals

HCB = Hexachlorobenzene

bis(n,n'-dimethyl) n,n'-dimethylbenzenamine, 1,5-naphthylenediamine, 4,4'-oxydianaline, pronetalol•HCl, 4,4'-thiodianaline, iodoform, dibromomethane and dichloroethane (Kraybill, 1983; Weisburger, 1983). Hexachlorobenzene is in a different chemical class from these agents.

Induction of thyroid tumors in the animal studies is of particular interest because a very high incidence of enlarged thyroids was found among victims of accidental exposure to hexachlorobenzene in Turkey (Peters, 1983). The incidence among females, over 25 years after the incident, is 61.4% whereas the background incidence in that geographic area for females is about 5% (Peters, 1983). The data and pathology reports have not been made available yet, but it is clear that the cohort exposed to hexachlorobenzene has an unexpectedly high incidence of enlarged thyroid. It cannot be stated at present what percentage if any of the enlarged thyroids is the result of tumorigenesis.

This hamster study provides strong positive evidence of tumorigenicity and evidence of carcinogenicity of hexachlorobenzene, as indicated by the significant increase in hepatomas, significant increase of thyroid adenomas in males and the occurrence of metastasizing liver haemangioendotheliomas in treated but not in control animals. Although not reported in detail in this one page publication, the authors noted an increase in adrenal neoplasms as well. The data presented show that the tumor incidence is positively dose-dependent in most instances and that this is true not only of the number of animals with tumors of all sites but also for the number of tumors per animal. The authors also indicated that latency period was reduced, but actual supporting data was not presented. Although strong evidence for carcinogenicity was provided in the hamster study, a cautionary note should be added regarding the results of this study and possibly other hexachlorobenzene studies as well. The hexachlorobenzene used was reported to be 99.5% pure. However, chlorinated dibenzofuran and chlorinated dibenzo-p-dioxin, both very potent carcinogens, have been reported in the past to be present in some samples of hexachlorobenzene. Very small amounts of such contaminants could influence results.

Lambrecht et al. (1982a) Hamster Study -- Another study on hamsters, carried out in a different laboratory, adds further suggestive evidence for the tumorigenicity of hexachlorobenzene in hamsters (Lambrecht et al., 1982a). This study, reported only in abstract form, was also carried out in the Syrian golden hamster. In this study the animals were exposed for only 90 days to the hexachlorobenzene. On day 91, half of the initial exposed 50 animals were sacrificed. The remaining animals were sacrificed periodically until the end of the 1-year study. The exposure levels used were 200 or 400 ppm hexachlorobenzene in the diet. Assuming that the hamsters from the Cabral (1977) study were comparable in weight and dietary consumption, these ppm figures would be approximately equal to and twice those of the high dose used in the lifetime studies of Cabral et al. (1977). Lambrecht et al. (1982a) reported the incidence of hepatoma at the 200 ppm level to be 7.7% in males and 6.7% in females; at the 400 ppm level the incidence was 5% in females and 14.3% in males. These figures are based on the numbers of animals at risk at the time of the earliest observed tumor. The time to first tumor was relatively late in the study, 276 days for males and 255 days for females of the lower dose and 153 days for males and 299 days for females at the higher dose. Since the test animals were systematically sacrificed from 3 months onward, the time to tumor figures should be reasonably close to actual time to tumor. Table V-3 shows the results reported by Lambrecht et al. (1982a).

The tumorigenicity and carcinogenicity of hexachlorobenzene has been demonstrated by one lifetime study in hamsters. Additional suggestive evidence for tumorigenicity is found in a 90-day study in another laboratory. In both cases hepatomas resulted. The longer period of exposure also produced thyroid adenomas and metastatic liver haemangioendothelioma.

Mouse Studies.

Cabral et al. (1979) -- Cabral et al. (1979) reported that outbred Swiss mice were fed hexachlorobenzene (99.5% purity) in their diets for up to 120 weeks. The hexachlorobenzene content of the diet was monitored periodically during the study and the diet was found to be free of aflatoxins. The exposure levels used were 50, 100 and 200 ppm corresponding to dosages of 6, 12 or 24 mg/kg/day based on body weight and food intake averages. One other test group was given 300 ppm (36 mg/kg/day) for only 15 weeks and retained on an hexachlorobenzene-free diet for the remainder of the study.

Growth rates were monitored but not given in detail in the published report. The investigators stated that among female mice there was a reduced growth rate for all doses except in the 12 mg/kg/day dose group and among males for all doses except in the 6 mg/kg/day dosage group.

Survival times were reported in detail. Survival was essentially unaffected in the two lower dosage level groups at 50 weeks, while at that time it was down by 60% of the original number in the females and 52% of the

TABLE V-3

Effect of HCB on Hamsters: Liver Tumors and Other Liver Lesions[a]

Sex	HCB (ppm)	PC+C[b] Incidence	BDH[c] Incidence	Day First Observed	Hepatomas Incidence	Day First Observed
M	0	3/50	0		0	
	200	48/49	0		1/13	276
	400	50/50	1/25	101	1/20	153
F	0	10/43	0		0	
	200	48/49	1/6	340	1/15	255
	400	45/45	2/20	174	1/7	299

[a] Source: Lambrecht et al., 1982a

[b] Precirrhotic + cirrhotic

[c] Biliary duct hyperplasia

HCB = Hexachlorobenzene

original number in the males in the highest dosage group. By 70 weeks on test the survival was decreased in the two lower dose groups as well, and in the highest dose group it was down to 14% in females and 10% in males. At 90 weeks there were only four surviving males out of the 50 and no surviving females in the highest dosage group as compared with 96 and 100% survival in the female and male controls.

The yield of tumors in this study is given in Tables V-4 and V-5. In Table V-4, the effective number of animals is the number of animals alive at the earliest time a liver cell tumor was observed in each group while in Table V-5 the effective number of animals is that number of animals alive at the earliest appearing tumor for any site in the body within that group. There was a statistically significant elevation in the incidence of liver cell tumors at the high dose in females and a marginal increase in high-dose males, with a positive dose-related trend in both cases. There was also a dose-dependent decrease in latent period and a dose-dependent increase in the size and multiplicity of liver cell tumors (see Table V-4). The liver cell tumors were subsequently defined as hepatomas (Cabral, 1983).

In this study there was a high incidence of both lymphoma and lung tumors in control mice. A dose-related decrease in the incidence of lymphomas appears in the treated groups. The investigators attributed this to the decreased survival time of hexachlorobenzene-treated animals. This seems reasonable but does not explain the reduction in lung tumors in the 50 ppm (6 mg/kg/day) group when they are compared to controls, since there was not an appreciable reduction of lifespan in this low dose group.

TABLE V-4

Liver Tumor Incidence in Mice Fed HCB[a]

Group	Effective[b] No. Animals	Mice with LCT		Node Size (mm)		Multiplicity		Age at Death (weeks)	
		No.	%	<8	≥8	Single	Multiple	Range	Average
HCB 100	F 12	3	25	2	1	1	2	87-104	98
	M 12	3	25	1	2	2	1	83-98	89
HCB 200	F 26	14	54	5	9	3	11	47-85	67
	M 29	7	24	4	3	2	5	46-101	73
HCB 300 (15 weeks exposure)	F 10	1	10	--	1	1	--	101	101
	M 3	1	33	--	1	--	1	97	97

[a] Source: Cabral et al., 1979

[b] Survivors at time first LCT was observed in each group

LCT = Liver cell tumors

HCB = Hexachlorobenzene

TABLE V-5

Tumor Data on Mice Fed HCB[a]

Group	Initial No. Animals	Effective[b] No. Animals	Animals with Tumors														
			TBA[c]		Lymphomas			Lung			Liver-cell		Gonads		Other	
			No.	%	No.	%	Average Age at Death (weeks)	No.	%	Average Age at Death (weeks)	No.	%	No.	%	No.	%
Control	F 50	49	39	80	21	43	89.6	14	29	89.0	0	0	3	6	9[d]	18
	M 50	47	22	47	12	26	80.8	13	28	83.8	0	0	0	0	4[e]	9
HCB 50	F 30	30	21	70	16	53	69.8	4	13	84.5	0	0	2	7	2[f]	7
	M 30	30	15	50	13	43	73.7	4	13	87.0	0	0	0	0	0	0
HCB 100	F 30	30	13	43	5	17	94.4	6	20	83.5	3	10	1	3	3[g]	10
	M 30	29	10	34	7	24	70.4	0	0	--	3	10	0	0	1[h]	3
HCB 200	F 50	41	19	46	5	12	58.2	2	5	66.5	14	34	1	2	1[i]	2
	M 50	44	12	27	4	9	53.2	4	9	82.5	7	16	1	2	0	0
HCB 300 (15 weeks)	F 30	26	20	77	8	31	97.7	4	15	91.2	1	4	3	12	8[j]	31
	M 30	16	5	31	3	19	68.6	2	13	83.5	1	6	0	0	0	0

[a] Source: Cabral et al., 1979

[b] Number of survivors at moment of appearance of first tumor at any site in each group

[c] In relation to the effective number

[d] Skin fibrosarcoma, uterine haemangioendothelioma, one skin haemangioendothelioma, two adrenal adenoma, two mammary adenoma

[e] Urinary bladder transition cell carcinoma, one liver haemangioendothelioma, one skin haemangioendothelioma, one skin fibrosarcoma

[f] One uterine haemangioendothelioma, one skin fibrosarcoma

[g] Two skin fibrosarcoma, one skin haemangioendothelioma

[h] One skin squamous-cell carcinoma

[i] One intestinal leiomyosarcoma

[j] One skin fibrosarcoma, two liver haemangioendothelioma, one cecum carcinoma, one stomach papilloma, one skin haemangioendothelioma, one uterine adenoma, one mammary adenoma

HCB = Hexachlorobenzene

This study by Cabral (1979) demonstrates the tumorigenicity of hexachlorobenzene in Swiss mice by the significant increase in liver cell tumors in both sexes and by the demonstration of dose-dependency in the response with respect to tumor incidence, tumor size, multiplicity and latent period duration. Tumorigenicity was detected as low as 12 mg/kg bw/day (100 ppm) for lifetime exposure but not at 6 mg/kg bw/day (50 ppm).

Lambrecht et al. (1982b) -- Swiss mice exposed to hexachlorobenzene for only 90 days at levels of 100 and 200 ppm in the diet showed degenerative changes of liver and kidneys when examined at various intervals after they were removed from the hexachlorobenzene-containing diet (Lambrecht et al., 1982b). Although liver tumors were not reported, treated animals showed lymphosarcomas in both dosage groups in both sexes at levels significantly above those of controls. Exposure to hexachlorobenzene in this instance produced leukemogenic changes. The animals were not permitted to live beyond selected intermediate sacrifice dates, so it was not possible to determine whether survivors would have developed liver or other tumors. The method of preparation of the hexachlorobenzene-containing diet may have been different in the Cabral et al. (1979) and Lambrecht et al. (1982b) studies, but detailed information was not presented in the Lambrecht et al. (1982b) abstract.

Mice may be somewhat less sensitive than hamsters to hexachlorobenzene as evidenced by the difference in incidence of hepatoma formation at various doses. These animal species may differ in the distribution of the hexachlorobenzene into various tissue compartments (Lambrecht et al., 1981), and differ in rates of metabolism and absorption. Administration of the same levels of hexachlorobenzene in the feed can be expected to give different effective dosages.

Shirai et al. (1978) -- Shirai et al. (1978) administered hexachlorobenzene to male ICR mice (35 animals/group) at levels of 10 or 50 ppm in the diet for periods of 24 weeks. Polychlorinated terphenyl was given alone to another group at 250 ppm, and in combination with 50 ppm hexachlorobenzene to a third group. Animals were examined histologically at 40 weeks.

Final body weights were slightly lower in the hexachlorobenzene-treated groups while liver weights were higher. Examination of the livers showed that the hexachlorobenzene-treated groups had hypertrophy of the centrilobular area at both doses. No liver tumors were found in either group. The total intake of hexachlorobenzene was calculated to be 8.4 and 35.3 mg/mouse over 24 weeks in the 10 ppm and 50 ppm groups, respectively.

Polychlorinated terphenyl alone, at 250 ppm (total dose 207.4 mg/mouse) gave 3/28 (10.7%) nodular hyperplasia. When this same level of polychlorinated terphenyl was given along with hexachlorobenzene at 50 ppm (total dose 36.9 mg/ mouse) there were 23/26 (88.5%) nodular hyperplasia and 8/26 (30.8%) hepatocellular carcinoma. This response indicates that hexachlorobenzene can enhance the carcinogenic potency of polychlorinated terphenyl.

The duration of administration, 24 weeks, in this mouse study and the doses used were below those used in the Cabral (1979) study on Swiss mice and also below the levels used in the 13-week study by Lambrecht (1982b) on Swiss mice. Therefore, it is not surprising that hepatomas were not found when hexachlorobenzene was given alone. The occurrence of liver lesions, however, does indicate the liver is a target organ.

These three studies in mice demonstrate the tumorigenicity of hexachlorobenzene with respect to the induction of hepatomas, the leukemogenic effect of subchronic exposure and the ability of hexachlorobenzene to enhance the carcinogenic effect of another compound.

Rat Studies.

Smith and Cabral (1980) -- The carcinogenic potential of hexachlorobenzene was tested in several different laboratories in rats. In one study (Smith and Cabral, 1980) small numbers of female Agus rats, and even smaller numbers of female Wistar rats, were used. There were 12 control and 14 treated Agus rats and 4 control and 6 treated Wistar rats. The hexachlorobenzene was analytical grade (99.5% purity) dissolved in arachis oil and mixed with the feed to give 100 ppm in the diet. This dietary level supplied an average daily dose of 6-8 mg/kg/day to the rats.

In this study the Agus rats showed signs of porphyria after 3 months exposure to hexachlorobenzene, but other toxic manifestations were not found. The investigators stated that "there was a steady decline in body weight to eventually 80% of control animals" (Table V-6). Examination of the weight data presented in the publication indicates that this interpretation is based upon comparison of "final" average weight in control (286±19 g) and treated (225±16 g) animals (see Table V-6), representing a 21% difference in weight. This method of comparison can be misleading since the final weights represent accumulated differences in growth rates and

TABLE V-6

Body Weights of Female Agus Rats Fed Hexachlorobenzene for 90 Weeks[a]

Weeks of Diet	Body Weight (g)		% Difference
	Control	HCB	
0	46 ± 6 (8)	45 ± 24 (9).	2
10	191 ± 5	180 ± 17	6
30	236 ± 13	212 ± 13[b]	10
50	257 ± 17	221 ± 19[c]	14
90	286 ± 19 (8)	225 ± 16 (7)[c]	21

[a] Source: Smith and Cabral, 1980

[b] Significantly different from controls as assessed by Student's t-test $p<0.01$

[c] $p<0.001$

Female Agus rats were fed HCB (100 ppm) in MRC 41B diet for 90 weeks and then killed. Weights are menas (no. of animals in parentheses) ± S.D.

HCB = Hexachlorobenzene

varying composition of the groups because of animal deaths. An effect produced, even transiently, at an early age, may persist in the figures, even though all subsequent growth may be normal. Growth rates, rather than absolute difference in weights provide a more suitable picture of the animal response. Growth rates for the time intervals reported were calculated based on the data given in the publication and are shown in Table V-7. The equation used was:

$$R = \frac{\text{weight at end of interval} - \text{weight at start of interval}}{\text{weight at start of interval}} \times 100$$

According to this calculation both groups of animals grew during each time interval.

The survival of the treated Agus rats was good; one test animal was sacrificed at 52 weeks and a second one died of pneumonia at 70 weeks. Both of these animals had liver cell tumors found by histologic examination. Another five treated animals were sacrificed at 75 weeks and the remaining seven treated animals lived until the end of the experiment at 90 weeks. Among controls, one was killed at 63 weeks and three more at 75 weeks. The remaining eight were killed at 90 weeks.

No control animals had liver pathology. In contrast, 14/14 (100%) of the treated Agus rats had liver tumors; the earliest of these was detected at 52 weeks. The livers of the treated animals were grossly enlarged and some of the tumors were 1.5-2 cm in diameter. Although one liver cell tumor was described as pedunculated, histopathology detail was not given, except to note the absence of metastases in all cases. Four of the six (67%) Wistar rats also had liver cell tumors and none of the four controls showed such pathology at 75 weeks.

TABLE V-7

Growth Rates for Female Agus Rats on a Diet Containing 100 ppm HCB*

Interval (on diet)	Average Growth Rate %/week	
	Control	Treated
0-10 weeks	31.5	30.0
10-30 weeks	1.2	0.89
30-50 weeks	0.45	0.22
50-90 weeks	0.28	0.05

*Source: Calculated from Smith and Cabral, 1980

HCB = Hexachlorobenzene

In this rat study hexachlorobenzene was a potent inducer of liver tumors, causing a 100% incidence with the earliest tumor observed at 52 weeks. It is important to determine whether the magnitude of the effect is all attributable to the hexachlorobenzene or whether contaminants, unusual characteristics of the test animals, or procedural factors were operative in this study. In this context the following points are noted.

First, historical control data on tumor incidence for Agus rats were not available, but, according to Cabral (1983), the Agus rat is a strain particularly sensitive to porphyria and hepatic tumors. In regard to the question of contaminants, peanut oil is generally believed to be free of aflatoxins [they are destroyed in processing (NAS, 1977)] and the feed was analyzed for both aflatoxins and dibenzofurans and found to be free of both (Cabral, 1983). Absorption is another factor to consider. The absorption of the hexachlorobenzene in these animals might be enhanced by dissolution in the arachis oil.

Lambrecht et al. (1983a,b, 1984) -- Another study on rats was carried out by Lambrecht et al. (1983a,b, 1984). In this study 94 Sprague-Dawley rats of each sex for each dosage and control groups were used. Four animals of each group were sacrificed at each of 10 intervals: 0, 1, 2, 3, 4, 16, 32, 48 and 64 weeks. The remaining 54 animals of each group were allowed to continue until they died, or to the end of the 2 years. The number of animals at risk was considered to be those that survived at least 12 months, since this was the earliest time to tumor. This number would be, at minimum, 54 plus some animals from the last sacrifice time.

The hexachlorobenzene was highly purified and the prepared diet monitored for hexachlorobenzene levels periodically. The preparation was also analyzed for aflatoxins and found to be negative. The test diet was prepared by mixing the hexachlorobenzene with dextrose and Wayne laboratory feed (1.5 g hexachlorobenzene + 98.5 g dextrose + 9.9 kg lab chow to give 150 ppm hexachlorobenzene). Half the amount of hexachlorobenzene was used in the mix for the 75 ppm hexachlorobenzene level. This oil-free vehicle is different from the vehicle used by both Smith and Cabral (1980) and Arnold et al. (1985). The hexachlorobenzene was well absorbed as demonstrated by progressive accumulation in fat which was measured in this study.

Based on an average food consumption of 22.6 g/rat/day for males and 16.5 g/rat/day for females, and on an average adult weight for females of 265 g and for males of 400 g, the low dose was calculated to be 4-5 mg/kg/day and the high dose, 8-9.5 mg/kg/day. In order to compare the results obtained in this study with those obtained in Sprague-Dawley rats by Arnold et al. (1985), more detailed calculation of doses at different time periods on test are given in Table V-8.

The administration of hexachlorobenzene in the diet at these doses in the Lambrecht et al. (1983a) chronic feeding study in rats resulted in liver pathology just before the appearance of hepatoma or hepatocellular carcinoma. Pathology observed at the early sacrifice time included parenchymal degeneration, preneoplastic foci and adenoma. At 48 and 64 weeks of the test females had gross liver tumors which measured between 1 and 2 mm^3. Porphyria was also detected.

TABLE V-8

Dosage Levels in the Chronic Feeding Study of Hexachlorobenzene in Sprague-Dawley Rats[a] (mg/kg/day)

Time on Diet[b] (weeks)	Males		Females	
	75 ppm	150 ppm	75 ppm	150 ppm
0	19.5	37.0	16.1	32.2
26	3.2	7.1	3.7	8.7
52[c]	3.3	6.4	3.8	8.0
79	3.4	6.7	3.5	8.4
99	6.2	10.0	4.3	10.6

[a] Source: Calculations and data provided by Lambrecht, 1984

[b] The animals were 3 weeks old when placed on test

[c] At 52 weeks on test the males consumed an average of 24.7 g of the diet/day and weighed an average of 553.7 g. The females consumed an average of 16.0 g diet/day and weighed an average of 311.7 g.

Rats that lived 12 months or longer showed a significant increase in hepatoma incidence in both sexes. A statistically significant increase in the incidence of hepatocellular carcinoma was found at both doses in the females, and in males a slight non-significant increase was found. None of the liver cell tumors metastasized. Table V-9 summarizes the findings.

Renal cell adenoma was found to be significantly elevated in both sexes but with greater frequency in males. In this study the control male group had a high incidence of renal cell adenoma which was not explained; nevertheless, the increase in the hexachlorobenzene-treated animals was statistically significant. The incidence of renal cell carcinoma in treated animals was not significantly increased over control animals in either males or females.

In an updated report from this laboratory (Peters et al., 1983) histopathology details were supplied. These data show that in addition to the liver and kidney lesions there was an increase in adrenal pheochromocytoma in female rats which was statistically significant at both 75 and 150 ppm. Females also had elevated incidences of adrenal cortical adenoma and hemangioma in the treated groups. Among males the background incidence of adrenal pheochromocytomas is high (76.5%), making it difficult to determine whether the 90.6% incidence found in the 150 ppm group has any biological significance. Other adrenal neoplastic and non-neoplastic lesions were detailed: hyperemia and/or congestion, cortical hyperplasia, preneoplastic foci, cysts, lipoma and adenocarcinoma; none of these were elevated in the treated animals. The adrenal tumor incidences are given in Table V-10.

TABLE V-9

Liver and Kidney Tumors in Sprague-Dawley Rats Given Hexachlorobenzene in the Diet for up to 2 years[a,b]

Exposure Level	Hepatoma		Hepatocellular Carcinoma		Renal Cell Adenoma		Renal Cell Carcinoma	
	M	F	M	F	M	F	M	F
0	0/54	0/52	0/54	0/52	7/54	1/52	0/54	1/52
percentage	0	0	0	0	13	2	0	2
75 ppm	10/52	26/56	3/52	36/56	41/52	7/56	0/52	2/56
percentage	19	46	6	64	79	13	0	4
150 ppm	11/56	35/55	4/56	48/55	42/56	15/54	0/56	2/54
percentage	20	64	7	87	75	28	0	4

[a] Source: Lambrecht et al., 1983a,b; Lambrecht, 1983

[b] The diet was prepared without solubilization of the hexachlorobenzene, but by mixing it as a pulverized solid.

TABLE V-10

Adrenal Tumors in Sprague-Dawley Rats Given Hexachlorobenzene in the Diet for up to 2 Years[a,b]

MALES

Days on diet	400-599			600+		
Exposure ppm hexachlorobenzene	0	75	150	0	75	150
Number of tissues examined	17	23	28	34	25	23
Cortical adenoma (%)	3	2	6	6	3	4
Pheochromocytoma (%)	3 (17.6)	6 (26.1)	9 (32.1)	26 (76.5)	17 (68)	21 (91.3)
Hemangioma (%)	0	0	0	0	0	0

FEMALES

Days on diet	400-599			600+		
Exposure ppm hexachlorobenzene	0	75	150	0	75	150
Number of tissues examined	12	5	13	35	47	32
Cortical adenoma (%)	0	3	2	2 (5.7)	11 (23.4)	6 (18.8)
Pheochromocytoma (%)	0	0	2	5 (14.3)	31 (66)	29 (90.6)
Hemangioma (%)	0	0	2	3 (8.5)	8 (17)	5 (15.6)

[a] Source: Peters et al., 1983

[b] The diet was prepared without solubilization of the hexachlorobenzene, but by mixing it as a pulverized solid.

One point to consider in the interpretation of the results, particularly in terms of their application to risk assessment, is the form in which the hexachlorobenzene was administered in the diet. The absorption from a particulate form introduces an additional possible exposure route, namely, from the food preparation by inhalation. This consideration does not invalidate the study, but raises the question of the actual exposure levels if an additional route of exposure was occurring in the same experiment simultaneously with oral ingestion. The affect of mixing the hexachlorobenzene in the diet in an oil free form may also affect absorption and thereby the effective dose.

Arnold et al. (1983) -- In this study hexachlorobenzene (99% pure) was administered to parental male and female Sprague-Dawley rats for 3 months. These animals were mated at that time and the females continued to receive hexachlorobenzene-containing diets during pregnancy and throughout lactation. At weaning, 50 pups of each sex were separated and fed for the remainder of their lifetime on hexachlorobenzene-containing diets. Controls were fed diets free of hexachlorobenzene. The range of doses used in this study is considerably lower than those used by either Smith and Cabral (1980) or Lambrecht et al. (1983a,b). Table V-11 shows the doses used in the Arnold et al. study at particular points in time since the doses were not adjusted throughout the study. These doses represent a greater exposure to the test animals from the point of view of exposure duration, since the F_1 animals were exposed *in utero* and during nursing in addition to their exposure from feeding on an hexachlorobenzene-containing diet. Total doses cannot be calculated since the actual dose received during nursing is not known.

TABLE V-11

Exposure Levels in the Chronic Feeding, 2-Generation Study of Hexachlorobenzene in Sprague-Dawley Rats[a]
(mg/kg/day)

Time on Diet[b] (weeks)	Exposure Level			
	0.32 ppm	1.6 ppm	8.0 ppm	40.0 ppm
		MALES		
1	0.04	--	0.93	4.85
30[c]	0.01	0.06	0.29	1.5
70	0.01	0.05	0.25	1.3
		FEMALES		
1	0.04	0.17	0.84	4.64
30[c]	0.02	0.08	0.40	1.9
70	0.01	0.06	0.32	1.6

[a] Source: Calculations and data provided by Arnold, 1984

[b] The animals were placed on feed at 6 weeks of age.

[c] The mean body weight of male controls was 663 g and for the highest dose group males 653 g. The mean weekly food consumption for male controls at that time was 178 g and for the highest dose group 169 g. Females of the same age weighed 351 g for controls and 353 g for the highest dose treated group and the mean weekly food consumption was 113 and 118 g, respectively.

Arnold et al. (1985) found no differences in treated F_1 animals when compared to controls with respect to growth rates, food consumption or hematology. The only observed difference was a decreased viability index for pups in the 40.0 ppm dose group.

Histopathology showed that F_1 females had a significant elevation in neoplastic liver nodules and in adrenal pheochromocytoma in the high dose females compared to controls (Table V-12). There was also a significant positive dose-related trend in the incidence of these tumors in F_1 females.

Among F_1 males, in the highest dose group parathyroid tumors were significantly increased: 25% (12/48) in the treated groups and 4.2% (2/48) among controls. Females also showed a few parathyroid tumors in the two highest dose groups but none in controls or in the two lowest dose groups. The differences were not significantly different from controls. Table V-12 gives the tumor incidences. Although kidney tumors were not reported to be elevated, there was an increased chronic nephrosis in the F_1 treated animals.

Arnold et al. (1985) -- In another study by Arnold et al. (1985) which was related to the 2-generation study, the effect of vitamin A, because of its supposed antitumorigenic properties, was tested in conjunction with hexachlorobenzene. This was a 1-generation study and the level of hexachlorobenzene was the same as the highest dose of the 2-generation study, 40 ppm. There were six separate groups of 50 animals each and the experiment ran for 119 weeks. At 29 weeks and at 49 weeks five animals from each group were sacrificed and evaluated histologically. The six groups are shown in Table V-13. The vitamin A did not apparently alter the effects of

TABLE V-12

Tumors in Organs that Showed Statistical Differences from Control in F_1 Sprague-Dawley Rats Treated with Hexachlorobenzene[a] [incidence (%)]

Dose at 30 weeks (mg/kg bw/day)	Parathyroid Adenoma		Adrenal Pheochromocytoma		Hepatocellular Carcinoma		Neoplastic Liver Nodules	
	Males	Females	Males	Females	Males	Females	Males	Females
Controls	2/48 (4.2)	0/49 (0)	10/48 (20.8)	2/49 (4.1)	0/48 (0)	0/49 (0)	2/48 (4.2)	0/49 (0)
0.01-0.02	4/48 (8.3)	0/49 (0)	12/48 (25.0)	4/49 (8.0)	2/48 (4.2)	0/49 (0)	0/48 (0)	0/49 (0)
0.06-0.08	2/48 (4.2)	0/50 (0)	7/48 (14.6)	4/50 (8.0)	1/48 (2.1)	0/49 (0)[b] 1/49 (2.0)[b]	0/48 (0)	2/50 (4.0)
0.29-0.40	1/49 (2.0)	1/49 (2.0)	13/49 (26.5)	4/49 (10.2)	3/49 (6.1)	0/50 (0)	2/49 (4.1)[b] 3/49 (6.1)[b]	2/49 (4.1)[b] 3/49 (6.1)[b]
1.5-1.9	12/49 (24.5)	2/49 (4.1)	17/49 (34.7)	17/49 (34.7)	0/49 (0)	0/49 (0)[b] 1/49 (2.0)[b]	1/49 (2.0)	10/49 (20.4)[b] 9/49 (18.4)[b]
Other statistical tests								
IARC trend test	$p \leq 0.01$	$p \leq 0.05$	$p \leq 0.01$	$p \leq 0.01$				$p \leq 0.01$
Armitage time-related trend test	$p \leq 0.01$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.01$				$p \leq 0.01$
Fisher exact treated vs. control	$p \leq 0.05$			$p \leq 0.01$				$p \leq 0.01$

[a] Source: Arnold et al., 1985; Arnold, 1984

[b] Different results of two different pathologists reading the same slides

TABLE V-13

Parathyroid and Adrenal Pheochromocytomas in Sprague-Dawley Rats Maintained on Synthetic Diets of Varying Vitamin A Content and With or Without Hexachlorobenzene*

Group	No. with Parathyroid Tumors	No. with Adrenal Pheochromocytoma
Controls on diet with normal vitamin A content	3	3
Control diet + 40 ppm HCB	4	6
Diet with 0.1 times normal vitamin A	0	2
Diet with 0.1 times normal vitamin A + 40 ppm HCB	0	2
Diet with 10X vitamin A	1	4
Diet with 10X vitamin A + 40 ppm HCB	3	7
Total without HCB	4	9
Total with HCB 40 ppm	7	15

*Source: Arnold et al., 1985

HCB = Hexachlorobenzene

hexachlorobenzene. The number of animals with parathyroid tumors and adrenal pheochromocytomas was somewhat elevated in all the cases in which hexachlorobenzene was administered compared with the total cases with the three levels of vitamin A and no hexachlorobenzene. The significance of these tumor incidences cannot be determined by simple comparison because it was also found in the study that vitamin A had an effect on the background level of some common tumors and these data have not yet been completely analyzed.

Discussion of Rat Studies. It seems appropriate to compare the findings of Smith and Cabral (1980) in Agus and Wistar rats, Lambrecht et al. (1983a,b) and Arnold et al. (1985) in Sprague-Dawley rats. None of the three studies agree precisely on all four of the tumor target organs: Smith and Cabral reported liver tumors, Lambrecht reported liver, adrenal and kidney tumors and had some liver carcinomas not found by Smith and Cabral. Arnold found adrenal and parathyroid tumors and neoplastic liver nodules but no increase in kidney tumors. We find that, although differences do occur, the results are not contradictory for the following reasons:

1. The dosages used in the Arnold et al. (1985) study were below those used by either Smith and Cabral (1980) or Lambrecht et al. (1983a,b). The range of doses used by Smith and Cabral was given as 6-8 mg/kg/day and those used by Lambrecht were 3-9 mg/kg bw/day. Those of Arnold were, at most, between 1.5 and 2.0 mg/kg bw/day.

2. There were notable differences in the animals used: in the case of Smith and Cabral the liver tumor susceptible strain of Agus rat was used, although tumors were also found with Wistar rats. We do not have full data on historical tumor incidences in these animals to allow for more detailed evaluation.

3. The conditions of the Smith and Cabral study and those of Lambrecht were both different from the 2-generation study of Arnold. Differences in sensitivity due to prenatal exposure may occur because of rapid cell division and/or differences in xenobiotic metabolism compared with older animals. The dose received transplacentally and from nursing is also uncertain.

4. The method of preparation of the hexachlorobenzene in the diet was different in that both Smith and Cabral and Arnold used arachis oil and corn oil as hexachlorobenzene solvents while Lambrecht did not use an oil vehicle. Absorption characteristics are known to depend upon the vehicles used.

5. The Sprague-Dawley animals used by Arnold may have more fat than those used by Lambrecht as they were somewhat larger. Distribution into different tissue compartments, especially into fat where it is likely the hexachlorobenzene is at least temporarily stored, is likely to alter the effective concentration in target tissues. In this regard the hexachlorobenzene is known to concentrate in adrenal tissue; the degree of such concentration may well vary with strain or diet of the host animals.

In summary, orally administered hexachlorobenzene has induced hepatocellular carcinoma in male Sprague-Dawley (S-D) rats as well as hepatomas in female Agus and Wistar rats and in S-D rats of both sexes. At the lowest dose used in any of the studies (40 ppm in the diet or 1.5 mg/kg/day), neoplastic nodules were induced in S-D rats, whereas hepatocellular carcinomas occurred in the same strain at a higher dose (4-5 mg/kg/day). Adrenal pheochromocytoma was significantly elevated in two separate studies in female S-D rats. In the same strain one investigator reported parathyroid tumors and a different investigator reported kidney tumors; neither of these findings has been repeated by other authors. Table V-14 summarizes this information.

Other Studies. In addition to the studies described on hamsters, mice and rats there are a few studies which cover specific kinds of tests other than lifetime exposure and examination of all potential target tissues for tumorigenic or carcinogenic response.

One such study was that of Theiss et al. (1977) in which the experiment was designed to detect only pulmonary tumors following i.p. injection of organic chemicals found as contaminants of drinking water. In this assay hexachlorobenzene was one of the chemicals tested. Strain A mice were given three dosage levels of hexachlorobenzene with the top level as the MTD. A total of 24 injections over a period of 8 weeks were given to 20 mice/group. The total doses received were 190, 480 and 960 mg/kg. The lungs were the only organ examined and hexachlorobenzene did not increase tumor incidence in that organ. The study ran for 32 weeks. Although this assay has proved useful in detecting some pulmonary carcinogens, it is not designed to detect other tumors.

TABLE V-14

Qualitative Comparison of Tumor Development in Rats Following Hexachlorobenzene Administration in Different Studies

Strain/Sex	Dosage (lowest dose that produced tumor)	Liver	Kidney	Adrenal	Parathyroid	Reference
Agus/Female	100 ppm (6-8 mg/kg bw/day)	liver-cell tumor (F)	NA	NA	NA	Smith and Cabral, 1980
Wistar/Female	prepared by dissolving in oil and mixing oil with food	liver-cell tumor (F)	NA	NA	NA	Smith and Cabral, 1980
Sprague-Dawley/ Male and female	75 ppm (3-4 mg/kg bw/day) prepared in feed sans oil vehicle	hepatocellular carcinoma (M&F) hepatoma (M&F)	renal cell adenoma (M&F)	pheochromocytoma (F) cortical adenoma (F)	NA	Lambrecht, 1983a,b
Sprague-Dawley/ Male and female F_1 animals of 2-generation study	40 ppm (0.3-1.5 mg/kg bw/day) prepared in oil and mixing oil with food at weaning -- animals exposed _in utero_ and during nursing	neoplastic liver nodules (F)	not found	pheochromocytoma (F)	adenoma (M)	Arnold, 1983

NA = It is not known whether or not these tissues were examined.

In another study on beagle dogs (Gralla et al., 1977) in which hexachlorobenzene was given in daily gelatin capsules to 30 animals of each sex/dosage group the duration of the study was only 1 year. Although this is not a long enough period of time for a carcinogenicity study in dogs, it is of interest to note that the doses of 100, 10, 1 and 0.1 mg/kg bw/day produced a number of toxic mani- festations in the liver including bile duct hyperplasia, hepatomegaly and liver necrosis. This study is more appropriately considered under chronic toxicity.

Finally, Pereira et al. (1982) designed a study to determine whether hexachlorobenzene increased γ-glutamyltranspeptidase-positive foci in rats. These foci are believed to be preneoplastic in the liver. The assay was designed to test initiation/promotion in this case by employing diethyl-N-nitrosamine (DENA) as the initiating agent and hexachlorobenzene as the promoter. Unfortunately, there are some errors in reporting of the results in the published paper and some important controls were not included (Pereira, 1983). We have not yet received a corrected manuscript.

Carcinogenicity Summary. In a lifetime study of hexachlorobenzene administration to hamsters, hepatoma was induced in both males and females. The response at a dose of 4-5 mg/kg/day dissolved in corn oil and mixed in the feed was 47% for both sexes and controls had no hepatomas. In addition to hepatomas, hamsters responded to hexachlorobenzene treatment with malignant liver haemangioendothelioma and thyroid adenoma. The incidence of haemangioendothelioma was 20% in males (versus 0 in controls) at 8 mg/kg/day and 12% in females (versus 0 in controls) at 16 mg/kg/day. The thyroid adenoma occurred at 14% incidence in males treated with 16 mg/kg hexachlorobenzene (versus 0 in controls).

Liver cell tumors, described as hepatomas, were also produced in both sexes in Swiss mice. At 24 mg/kg/day the incidence was 34% for females and 16% for males and the response showed a dose-dependency not only in the number of tumor-bearing animals but also in the latent period, multiplicity and size of tumors. In ICR mice, hexachlorobenzene administered concurrently with polychlorinated terphenyl induced hepatocellular carcinomas.

In rats target organs for hexachlorobenzene-induced tumors included liver, kidney, adrenal gland and parathyroid gland in various studies. Liver tumors were found in three studies which included three different strains of rat: Agus (a liver tumor sensitive strain), Wistar and Sprague-Dawley rats. These tumors were induced with doses between 1.5 and 8 mg/kg/day. The incidence was as high as 100% in Agus rats but lower for the other strains. Renal cell tumors were found in one study on Sprague-Dawley rats. In two studies on Sprague-Dawley rats, significant increases in adrenal pheochromocytoma in females were found. In one of these studies the incidence of parathyroid tumors in males was significantly increased as well.

Table V-15 summarizes the tumor data for hamsters, mice and rats for hexachlorobenzene experiments.

The data on hexachlorobenzene provide sufficient evidence of the carcinogenicity and tumorigenicity of hexachlorobenzene since there were increased incidences of malignant tumors of the liver in two species (haemangioendothelioma in hamsters and hepatocellular carcinoma in rats) as well as reports of hepatoma in mice, rats and hamsters.

TABLE V-15

Significantly Increased Incidence of Tumors in Animals Given Hexachlorobenzene in Diet

Animal (strain)	Organ	Tumor	% Treated/% Control		Lowest Dose to Produce Tumor (mg/kg bw/day)	Reference
			Males	Females		
Hamsters	liver	hepatoma	47/0	47/0	4	Cabral, 1977
Hamsters	liver	haemangioendothelioma	20/0	12/0	8 in males 16 in females	Cabral, 1977
Mice	liver	hepatoma	16/0	34/0	24	Cabral, 1979
Rats (S.D.)	liver	neoplastic nodules	NS	20/0	1.5	Arnold, 1983
Rats (S.D.)	liver	hepatoma	19/0	46/0	4-5	Lambrecht et al., 1983a
Rats (S.D.)	liver	hepatocellular carcinoma	NS	64/0	4-5	Lambrecht et al., 1983a
Rats (Wistar)	liver	hepatoma		67/0	6-8	Smith and Cabral, 1980
Rats (Agus)	liver	hepatoma		100/0	6-8	Smith and Cabral, 1980
Rats (S.D.)	adrenal	pheochromocytoma	NS	35/4	1.5	Arnold, 1983, 1984
Rats (S.D.)	adrenal	pheochromocytoma	NS	91/14	4-5	Peters et al., 1983
Rats (S.D.)	kidney	renal cell adenoma	79/13	13/2	4-5	Lambrecht et al., 1983b
Rats (S.D.)	parathyroid	adenoma	25/4	NS	1.5	Arnold, 1983
Hamsters	thyroid	adenoma	14/0		16	Cabral, 1977

NS = Not stated

The appearance of thyroid tumors in hamsters and adrenal pheochromocytomas and parathyroid tumors in rats as a result of hexachlorobenzene exposure is particularly interesting because of the clinical association of adrenal pheochromocytomas with parathyroid and thyroid tumors in humans (Fraumeni, 1974; Hill, 1974), and because follow-up of individuals in Turkey, who were accidentally exposed to hexachlorobenzene over 25 years ago, shows a marked elevation in thyroid tumors. Only a few of these subjects have had their thyroid tumors examined histologically and the pathology reports are not yet available.

If the IARC criteria for the classification of carcinogens were used, this animal evidence would be considered "sufficient." In the absence of human evidence of carcinogenicity, hexachlorobenzene would be classed in IARC category 2B, meaning that it has been demonstrated to be carcinogenic in animals and is probably carcinogenic in humans.

Reproductive and Teratogenic Effects

Hexachlorobenzene has been shown to be cross the placenta into fetal tissues and to be present in the milk of nursing dams (see Chapter III). The NOEL in a 4-generation reproduction study with rats was reported to be 20 ppm of hexachlorobenzene in the diet. Pups from treated dams (receiving diets containing 80 ppm hexachlorobenzene) recovered from elevated liver weights when nursed by foster dams. Hepatomegaly and reduced survival was reported in kittens from cats receiving 263 ppm of hexachlorobenzene in their diets. Infant rhesus monkeys developed clinical signs of toxicity, but histologic examination showed only mild effects. Fetal mice from dams treated with 100 mg/kg/day during days 7-16 of gestation exhibited teratogenic abnormalities.

Results from a 4-generation reproduction study with Sprague-Dawley rats was reported by Grant et al. (1977). Weanling rats, in groups of 20 females and 10 males, were fed diets containing 0, 10, 20, 40, 80, 160, 320 or 640 µg hexachlorobenzene/g and at 100 days of age the F_0 generation was mated to produce the F_{1a} generation. The F_{1a} pups were weaned at 21 days, and the F_0 rats were rested for 14 days and again mated to produce the second litter, F_{1b} animals. The F_{1b} animals were then used to produce the next generation, and this sequence was followed to the F_{4b} generation. The two highest doses (320 and 640 µg/g) were toxic to the mothers and resulted in 20 and 50% mortality, respectively, before the first whelping and 25% in each high dose group before the second whelping. In addition, the fertility index in these rats was greatly reduced in these two dose groups and the average litter size was decreased in the F_{1b}, F_{2a} and F_{2b} generations. The pups exhibited no gross abnormalities, but there was an increased number of stillbirths and all pups born alive died within 5 days in the 320 and 640 µg/g diet groups.

At the 160 µg/g level, 55% of the pups survived to day 5 but survival to day 21 was greatly reduced. The number of live births and survival was normal for the first two generations at the 80 µg/g dietary level, but by the third generation there were stillbirths and a low degree of postnatal viability. In addition, birth and weanling body weights were consistently less than those of the control group. At 40 µg/g diet only the liver weights of the 21-day-old pups were significantly increased, while the kidney, heart and brain weights were not affected. Tissue concentrations of hexachlorobenzene were dose-related, with body fat having the highest concentration. The NOEL was reported to be 20 ppm in the diet.

The effect of hexachlorobenzene on rat reproduction was also reported by Kitchin et al. (1982). Female Sprague-Dawley rats (10 animals/treatment group) were fed diets containing 0, 60, 80, 100, 120 and 140 µg hexachlorobenzene/g of diet. The females were mated with untreated males after 96 days and then bred a second time 12 days after weaning of the F_{1a} litter. Fertility and fecundity of treated females were not affected by treatment; however, a dose-related 21-day increase in mortality was observed in both litters and the LD_{50} values were determined to be 100 and 140 µg/g (maternal dietary concentration) for the F_{1a} and F_{1b} generation, respectively.

Mendoza et al. (1978) studied the effects of hexachlorobenzene on preweanling Wistar rats after a reciprocal transfer between 5 treated and 5 control dams. A significant increase in the liver weight over that of the control was observed in pups nursed by dams fed diets containing 80 µg hexachlorobenzene/g for 2 weeks before mating until birth, but this effect did not persist after the treated pups were transferred to a control foster dam. Similarly, the pups nursed by treated dams had smaller brains, hearts, kidneys and spleens than the controls, and these organs were larger in treated pups nursed by control dams. The authors concluded that hexachlorobenzene transmission via the milk had greater effects on the pups than transmission via the placenta.

Mendoza et al. (1979) placed female Wistar rats on diets containing 80 µg hexachlorobenzene/g beginning 2 weeks before mating until 35-36 days after weaning. Results indicated that there were no marked differences in the external appearance, body weight, liver weight, gestation, or neonatal survival between the hexachlorobenzene treated and control females. In addition, there were no differences in the number of litters, average number of pups/litter, average number of pups at birth and gestation index.

Hansen et al. (1979) studied the effects of hexachlorobenzene on reproduction in cats fed contaminated pork cakes for 142 days. These cakes contained 90±51 µg hexachlorobenzene/g, equivalent to an intake of 3 mg/day/cat, and were obtained from gilts fed diets containing 100 µg hexachlorobenzene/g for 6-8 weeks before slaughter. The positive and untreated control groups received pork cakes from gilts fed diets that did not contain hexachlorobenzene, with the positive control group receiving hexachlorobenzene-spiked cakes (263±120 µg/g equivalent to 8.7 mg/day/cat). These females were mated with untreated males and the resulting kittens did not receive hexachlorobenzene-containing cakes. Effects on survival were noted in kittens born to only those cats receiving hexachlorobenzene-spiked cakes and was apparently due to the kittens being too weak to survive the stress of weaning. There was a tendency for reduced average litter sizes and increased mortality of nursing positive control kittens, and statistically significant hepatomegaly and reduction in positive control kitten survival at weaning. Treated positive control females exhibited a net weight loss and increased susceptibility to disease but no changes in relative organ weights, hematologic parameters, or fecal coproporphyrin excretion.

Rush et al. (1983) fed adult male and female standard dark minks (Mustela vision) diets containing 0, 1 or 5 ppm hexachlorobenzene and then mated the males to the females in each of the respective study groups. The resulting mink kits were fed their parents respective diets after weaning from their mothers. The effects of exposures to hexachlorobenzene in utero and from nursing milk resulted in increased mortality in the hexachlorobenzene-treated weanlings with mortality in the 0, 1 and 5 ppm groups being 8.2, 44.1 and 77.4%, respectively. The surviving kits from all three groups had no observed alterations in whole body, kidney or liver weights and no observed damage to the kidneys or livers at 17 weeks of age. Induction of hepatic mixed-function oxidases was observed in the surviving hexachlorobenzene-exposed kits without any observable frank hepatotoxicity.

Bailey et al. (1980) studied the transfer of hexachlorobenzene to three nursing infant rhesus monkeys from three lactating mothers receiving by gavage 64 mg/kg/day of hexachlorobenzene suspended in methyl cellulose for 60 days. The hexachlorobenzene concentrated in the mothers' milk ranged from 7.51-186 ppm during the dosing schedule. One infant, by day 22, had developed symptoms of hypoactivity and lethargy which progressed to ataxia and death 1 week later. Autopsy revealed severely congested lungs. A second infant died on day 38 and autopsy revealed a subdural hematoma and bilateral hemorrhagic pneumonia. This indicated that the risk of exposure to nursing infants was greater than the risk to their mothers. Blood (0.42-49.44 ppm) and tissue levels in the infants were higher than in their mothers (0.41-16.16 ppm blood), and the infants developed clinical symptoms of toxicity while the mothers were asymptomatic.

Studies on the placental transfer of hexachlorobenzene in Wistar rats and New Zealand rabbits did not reveal any apparent adverse effects on fetal development. The female rats were dosed daily with 5, 10, 20, 40 or 80 mg/kg from day 6-16 of gestation, whereas the rabbits were treated with 0, 0.1, 1.0 or 10 mg/kg from day 1-27 of gestation. The compound was dissolved in corn oil and administered by means of a stomach tube (Villeneuve et al., 1974; Villeneuve and Hierlihy, 1975).

Khera (1974) conducted a teratogenicity study with groups of 7-16 female Wistar rats given single oral doses of 0, 10, 20, 40, 60, 80 or 120 mg hexachlorobenzene/kg suspended in corn oil or 0.25% aqueous gum tragacanth during gestation days 6-21. Maternal toxicity and reduction in fetal weights resulted from the two higher doses. Maternal toxicity was characterized by loss in body weight, hyperesthesia, tremors and convulsions. A significant increase in the incidence of unilateral and bilateral 14th rib was observed and was related to the duration of treatment (days 10-13, 6-16 or 6-21 of gestation) and the dose. Sternal defects were observed in only 1 of 4 experiments, which lead the authors to conclude that it was doubtful that hexachlorobenzene caused the observed sternal defects. There were no hexachlorobenzene-related effects on external morphology. Visceral abnormalities were not observed, and microscopic examinations did not reveal any treatment-related change in the histology of the fetuses. Values for live and dead fetuses, resorption sites, and fetal weight were within the control limits.

Courtney et al. (1976) studied the effects of ingestion of 100 mg/kg/day hexachlorobenzene on days 7-16 of gestation in 10 pregnant CD-1 mice. This study was undertaken to evaluate the possibility that hexachlorobenzene could be responsible for fetal malformations seen in pregnant animals exposed to hexachlorobenzene-contaminated pentachloronitrobenzene. The results showed that the hexachlorobenzene-treated mice had significantly increased maternal liver-to-body weight ratios and decreased fetal body weights. Also, a significant increase in the incidence of abnormal fetuses per litter were observed as compared to control mice. The abnormalities that were observed in these affected fetuses were cleft palates, one straight leg, small kidneys, one renal agenesis, and enlarged renal pelvis. They concluded from this study that the teratogenic activity of contaminated pentachloronitrobenzene was probably due to hexachlorobenzene.

Summary

The acute oral toxicity of hexachlorobenzene has been found to be low, with LD_{50} values ranging from 1700-10,000 mg/kg. Subchronic oral toxicity studies with a number of mammalian species indicated a significant increase in liver and kidney weights in hexachlorobenzene-treated animals. Some studies have shown increases in other organs as well. The livers from hexachlorobenzene-exposed animals have shown histologic changes such as irregular shaped and moderately enlarged liver mitochondria and increases in the size of the centrilobular hepatocytes. Chronic oral toxicity studies revealed similar effects to those seen in the subchronic studies plus hexachlorobenzene-associated life-shortening and various hepatic and renal pathologies. These subchronic and chronic effects were usually dose-related. Other effects included multiple alopecia and scabbing, together with neurologic effects in rats, mice and dogs. A dose-related histopathologic change in the ovaries of monkeys has also been reported.

Increased porphyrin levels in the liver and in urine have been reported for all species studied except the dog, which does not exhibit increased porphyrin levels. Hexachlorobenzene was found to cause the accumulation of β-H-steroids which induce porphyrin biosynthesis and to inhibit uroporphyrinogen decarboxylases. The inhibition of uroporphyrinogen decarboxylases appears to be due to pentachlorophenol, a hexachlorobenzene metabolite. Indications are that females are more susceptible to hexachlorobenzene-induced porphyria than are males, which may be related to the female estrogen levels and greater hexachlorobenzene metabolism. Hexachlorobenzene was reported to produce a mixed-type induction of cytochromes resembling that produced by a combination of phenobarbital (P-450) and 3,4-benzpyrene (P-448). In addition, the activities of several hepatic microsomal enzymes were found to be induced by hexachlorobenzene.

Hexachlorobenzene did not induce dominant lethal mutations in two studies but was reported to be mutagenic in a yeast, S. cerevisiae, assay at a concentration of 100 ppm. Hexachlorobenzene possessed no detectable levels of mutagenic activity in the Salmonella histidine reversion assay. The chronic toxicity studies provide sufficient evidence of the carcinogenicity of hexachlorobenzene in animals since there was an increased incidence of malignant tumors of the liver in two species, haemangioendothelioma in hamsters and hepatocellular carcinoma in rats as well as confirmed reports of hepatoma in both of these species. Hexachlorobenzene was found to cause teratogenic effects in fetal mice whose mothers were ingesting 100 mg/kg/day of hexachlorobenzene during days 7-16 of gestation.

VI. HEALTH EFFECTS IN HUMANS

The effects of hexachlorobenzene on humans as a result of accidental or occupational exposure have been reviewed by Courtney (1979) and Currier et al. (1980). A few reports of data collected on occupationally exposed workers have been reported with studies conducted in Turkey and in the United States (i.e., Louisiana) on the general population following accidental exposure to hexachlorobenzene. The exposure of humans to toxicologically significant levels of hexachlorobenzene in Turkey from 1955-1959 by ingestion of contaminated grain, as reported by Cam (1959, 1960), Cam and Nigogosyan (1963) and Peters (1966), caused an epidemic of hexachlorobenzene-induced porphyria cutanea tarda (PCT), also known as porphyria turcica.

Epidemiologic Studies

Burns et al. (1974) found 0-310 ppb hexachlorobenzene in blood samples from 20 vegetable spraymen. There were no signs of PCT, and no correlations were observed between hexachlorobenzene levels and urinary porphyrin excretion, serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase or lactate dehydrogenase. Increased levels of urinary porphyrins were detected in 1 of 54 men occupationally exposed to hexachlorobenzene (Morley et al., 1973).

A medical survey was conducted by Dow Chemical Company (Currier et al., 1980) on 50 employees working at a chlorinated solvents plant in Louisiana, to determine blood hexachlorobenzene levels and signs suggestive of PCT or other adverse effects, as well as any changes in hematologic, clinical chemistry and urinalysis parameters. The results from this study are of limited value because the various parameters studied during the 4-year period were analyzed by several laboratories using various methods and on different individuals. There was potential exposure to other substances also. During various times of the study, the time-weighted-average airborne concentrations of hexachlorobenzene ranged from <1-13 ppb, and wipe samples from surfaces in the control, laboratory and clerical work areas ranged from 0.03-1.24 μg/100 cm^2.

The laboratory analyses and physical examinations performed on the 1977 study group and on a control group from a polyethylene plant did not reveal any signs indicative of PCT. Levels of hexachlorobenzene, urinary porphyrin and coproporphyrin and the average years of exposure are listed in Table VI-1. A statistically significant ($p<0.05$) correlation was found between hexachlorobenzene levels in blood and the number of years worked in the plant. For the other studied parameters no statistically significant differences were noted between the 44 chlorinated solvents workers and the 44 control workers for 1977, except for higher protein levels and lower hematocrit values in the former workers which were not considered to be biologically significant. In addition, significantly lower levels of urinary coproporphyrins and albumin were detected in white male workers with hexachlorobenzene blood levels ≥200 ppb than in those with hexachlorobenzene levels <200 ppb.

Burns and Miller (1975) studied plasma hexachlorobenzene residues of 86 residents living and/or working in an area exposed to the production, transportation and disposal of "hex" wastes (hexachlorobenzene and other chlorinated hydrocarbons) in Louisiana. Plasma hexachlorobenzene levels were

TABLE VI-1

Results of Blood and Urine Analysis in Men Employed in a Chlorinated Solvents Plant, 1974-1977^{a}

Parameter	Study Group				Comparison Group
	1974 (n=50)	1975 (n=49)	1976 (n=49)	1977 (n=44)	1977 (n=44)
Blood HCB (µg/ℓ)	310.7 ± 287.7^{b}	311.5 ± 242.9^{b}	159.9 ± 142.7^{c}	170.3 ± 111.8^{c}	0.1 ± 0.6
Urinary uroporphyrins (µg/ℓ)	22.4 ± 21.1	20.9 ± 11.0	37.4 ± 14.4	26.2 ± 14.3	NR
Urinary coproporphyrins (µg/ℓ)	77.4 ± 40.5	67.2 ± 36.1	100.6 ± 40.8	95.2 ± 48.9	NR
Age (years)	30.1 ± 6.3	31.1 ± 6.6	30.8 ± 6.7	31.7 ± 7.1	31.3 ± 6.8
Plant-years	5.5 ± 3.9	6.3 ± 4.0	5.9 ± 4.5	6.6 ± 4.8	6.6 ± 4.4

[a] Source: Currier et al., 1980; 1974-1975 results conducted by Bioscience Laboratories; 1976-1977 results conducted by Pathology Laboratories (± Standard Deviation)

[b] In plasma

[c] In blood

N = Sample size

NR = Not reported

HCB = Hexachlorobenzene

measured and correlated with demographic characteristics, occupational hazards, food sample analyses and house dust analyses. Average plasma levels of hexachlorobenzene ranged from 2.4-3.6 ppb in exposed subjects as compared with 0.5 ppb in controls ($p<0.001$; Table VI-2).

Higher levels of hexachlorobenzene residues, which were statistically significant ($p<0.05$), were found in the male subjects (4.71 ppb) than in the female subjects (2.79 ppb). These were not associated with race or exposure to hexachlorobenzene through the consumption of homegrown vegetables and animals. About 68% of the house dust samples contained an average hexachlorobenzene concentration of 380 ppb as compared with 20 ppb in control samples. When the hexachlorobenzene levels in dust were compared with the mean plasma hexachlorobenzene residues for the same household, a significant correlation was obtained ($p<0.025$). In addition, blood samples from 11 workers employed for an average of 4.8 years (10 months to 15 years) at the chemical plant contained an average of 78.6 (14-233) ppb hexachlorobenzene.

Accidental Ingestion in Turkey

The hexachlorobenzene-induced PCT epidemic in Turkey, a result of exposure during 1955-1959 in individuals who used contaminated seed wheat for food, has been reviewed by Courtney (1979). Cam and Nigogosyan (1963) estimated that 0.05-0.2 g of hexachlorobenzene was consumed per day. The method of estimation was not described. PCT is a disease of disturbed porphyrin metabolism manifested by cutaneous lesions and is commonly followed by hypertrichosis (hairiness) and hyperpigmentation. The induction of porphyria by hexachlorobenzene has been reviewed (DeMatteis, 1967; Granick, 1965; Tschudy and Bonkowsky, 1972; Courtney, 1979). Porphyrias are

TABLE VI-2

HCB Plasma Levels in Exposed Individuals and Controls[a]

Parameter	Exposed[b]	Controls[b]
Number of subjects	86	43
Age (years)	39.8 ± 19.1	32.3 ± 18.6
Black/white ratio	1.0	2.3
HCB plasma residues (ppb)	2.4 ± 2.3[c]	0.5
Range (ppb)	0-23	0-1.8
Percent positive	99	95
Percent >1 ppb	99	5

[a] Source: Burns and Miller, 1975

[b] Values are mean ± 1 SD

[c] Level for random sample only, N=63 (3.6 ± 4.3 for random and biased samples, N=83)

HCB = Hexaclorobenzene

metabolic disorders of porphyrin metabolism that are characterized by increased excretion of porphyrins and their precursors. Normally, δ-aminolevulinic acid synthetase is the rate-limiting step in porphyrin synthesis and heme acts as an end-product inhibitor or an end-product repressor of δ-aminolevulinic acid synthetase. In hexachlorobenzene-induced porphyria, δ-aminolevulinic acid synthetase is induced but heme does not suppress or inhibit the enzyme. The activity of uroporphyrinogen decarboxylase is decreased, consequently, porphyrin and its precursors (e.g., uroporphyrinogen, coproporphyrinogen and occasionally series I porphyrins) are excreted mainly in the urine but also in the feces. Increased levels of porphyrins also can be measured in the liver, skin, intestinal tract and other tissues (Courtney, 1979). PCT appeared to occur more frequently in children 4-16 years of age, whereas the number of adults and children under 5 years of age reporting PCT was much lower (10-24% of cases were individuals over 15 years of age and <5% were children below the age of 4). A distinct disease described as "pink sore" was observed in children under 1 year of age and achieved an epidemic scale. The clinical symptoms were weakness and convulsions and usually death in children whose mothers had clinical symptoms of PCT or who had ingested contaminated bread during gestation and/or lactation. The presence of hexachlorobenzene in the milk of nursing mothers suggested that pink sore was a manifestation of hexachlorobenzene toxicity. The reviewer states that there was a 95% mortality in these infants in addition to the very high incidence of stillbirths.

In a follow-up study, Cripps et al. (1981) examined 32 patients 20 years after the onset. Porphyrins were determined in urine and stool specimens of

29 patients and clinically significant porphyrin levels were observed in 5 patients. Clinical features such as hyperpigmentation, scarring, pinched facies, hypertrichosis, enlarged thyroid and distinctive arthritis were present in about half of the patients.

A detailed follow-up study was also conducted with 161 Turkish patients 25 years after the initial hexachlorobenzene incident (Peters et al., 1982). The patient group studied included some of the patients previously examined (Peters et al., 1966). Twenty-six patients were over 17 years of age at the time of acute toxicity, whereas the average age of the remaining patients was 7.1 years. An evaluation of the clinical signs and symptoms is summarized in Table VI-3.

The chronic disease state was manifested by generalized hyperpigmentation and hypertrichosis, scarring on the cheeks and hands, and tight sclerodermoid changes of the nose with perioral scarring. The most striking clinical features in those patients who developed signs of hexachlorobenzene toxicity at an average age of 7 years consisted of painless arthritic changes with osteoporosis of carpal, metacarpal and phalangeal bones and atrophy or failure to develop in the terminal phalanges. In addition, neurologic symptoms including weakness, paresthesias, myotonia, cogwheeling and painless arthritic changes of the hands and feet, were observed in 50-70% of the patients examined. Since the signs and symptoms 20-25 years later represented a continuum of signs and symptoms observed personally by Peters and Gocmen (1959-1963), it was concluded that the symptoms represented the effects of both hexachlorobenzene toxicity and changes caused by the induced mixed porphyria. Control patients from the villages inhabited

TABLE VI-3

Clinical Signs and Symptoms in Humans 25 Years After Exposure to Low Levels in HCB in Turkey, 1955-1959[a]

Clinical Signs/Symptoms	No. of Patients with Symptoms[b]	Percent
Porphyria--Neurological		
Weakness	117 (161)	73
Paresthesias	89 (161)	55
Sensory shading	75 (125)	60
Nervousness	39 (60)	65
"Cogwheeling"	35 (76)	46
Colic	34 (125)	27
Constipation	84 (161)	52
Recent red urine	31 (161)	19
Enlarged liver	17 (161)	11
	10 (161)	6
Dermatologic		
Hyperpigmentation	125 (161)	78
Scarring	134 (161)	83
Hirsutism	81 (161)	50
Pinched facies	69 (161)	43
Fragile skin	62 (161)	39
Thyroid enlargement		
Total	64 (161)	40
Men	26 (98)	27
Women	38 (63)	60
Orthopedic and others		
Arthritis	108 (161)	67
Small hands	107 (161)	67
Short stature	74 (161)	46

[a] Source: Peters et al., 1982

[b] Numbers in parentheses represent total number of patients examined for this symptom

HCB = Hexachlorobenzene

by these patients included unaffected family members and clearly demonstrated clearly the uniqueness of this disorder which allowed for ready identification of affected patients. In addition the 60% incidence of large thyroid tumors in the females proved a sharp contrast to the 5% incidence of thyroid tumors in the geographical area. No conclusions were drawn as to the incidence of cancer and mortality. Studies on these endpoints are still in progress and the length of time that has elapsed from the time of exposure may not yet be adequate for drawing conclusions.

A boy and three women of the exposed individuals treated in the early 1960's with i.v. and/or oral edetic acid (the metal chelating agent EDTA) showed no active symptoms when examined, and skin pigmentation and scarring were much less severe than in most of the other patients. Urine and/or stool porphyrin studies showed that seven patients had clearly recognizable increases in porphyrin levels (Table VI-4). Clinical chemistry and milk residue data are summarized in Table VI-5. Percent δ-aminolevulinic acid values were found to be above the upper normal limit of 4 mg/ℓ in 32 of 55 patients. The average residue levels in human milk samples from Turkish mothers with porphyria was 0.51±0.75 ppm; 0.16±0.23 ppm was found in milk samples from nonporphyric but hexachlorobenzene-exposed mothers.

Summary

A few epidemiologic studies with occupationally-exposed workers have been reported, together with studies and surveys conducted in Turkey and in the United States (i.e., Louisana), on the general population following accidental exposure to hexachlorobenzene. These studies qualitatively support the toxicity of hexachlorobenzene but give little dose response

TABLE VI-4

Porphyrin Levels in Patients and Controls*

	Stool (µg/g dry weight			Urine (µg/l)	
	Coproporphyrin	Protoporphyrin	Uroporphyrin	Coproporphyrin	Uroporphyrin
Controls					
Turkey, mean ± SD (N=33)	4.80 ± 3.2	7.65 ± 9.83	1.41 ± 1.57	30.0 ± 23.6	5.80 ± 4.25
United States, mean ± SD (N=40)	6.1 ± 4.7	21.1 ± 11.6	2.8 ± 2.7	69.0 ± 27.0	9.0 ± 4.0
Hexachlorobenzene-Exposed Patients					
Patients with active porphyria (N=15)	70.14 (1.0-837.6)	12.19 (0.7-61.8)	25.8 (0.7-189.2)	174.5 (32.6-779.3)	111.4 (16-1607)
Remainder (N=146)	5.74 (0.5-4.1)	9.02 (0-103.4)	1.19 (0-12.6)	31.91 (0-198.4)	7.25 (0-29.5)

*Source: Peters et al., 1982

TABLE VI-5

Laboratory Test Results of Turkish Patients[a]

Test	Normal Range	Patient Range	No. of Abnormal Results[b]
Urine			
δ-Aminolevulinic acid, mg/ℓ	<4	0.14-10.1	32 (55)
Porphobilinogen, mg/ℓ	<1	0.11-1.04	0 (56)
Copper, ppm	0.01-0.06	0.01-0.046	0 (31)
Zinc, ppm	0.1-0.7	0.02-1.22	7 (31)
Serum			
Copper, µg/dℓ	70-155	88-153	0 (30)
Zinc, µg/dℓ	70-120	57-112	9 (29)
Creatine kinase, units/ℓ	women, <120	65-141	1 (8)
	men, <150	51-318	4 (11)
Iron, µg/dℓ	65-170	69-147	0 (29)
Thyroid function tests	5-11	2.2-10.1	women, 5 (10)
Thyroxine, µg/dℓ			men, 2 (9)
Triiodothyronine uptake, percent	37-59	36-51.1	women, 1 (10)
			men, 1 (9)
Free thyroxine index	1.85-6.5	0.9-4.6	women, 4 (10)
			men, 0 (9)
Blood			
Lead, erythrocyte, µg/dℓ	<35	2-17	0 (11)
Uroporphyrinogen synthetase[c]	>20	12.4-34.8	5 (30)
Milk hexachlorobenzene, ppm[d]			
Patients with porphyria	NA	0.51 (0-3.12)	53 (56)
Patients without porphyria	NA	0.16 (0-1.26)	16 (77)

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[a] Source: Peters, et al., 1982

[b] Numbers in parentheses represent total number of patient specimens analyzed.

[c] Values expressed in nanomoles formed per milliliter of RBCs per hour

[d] Allowable limit in United States for cow's milk is 0.02 ppm

NA = Not applicable

information. Biological monitoring of plasma levels show clearly more hexachlorobenzene in plasma of exposed compared to non-exposed individuals although no biologically significant adverse health effects were seen during the observation periods.

The exposure of humans to hexachlorobenzene in Turkey from 1955-1959 caused an epidemic of hexachlorobenzene induced PCT, also known as porphrya turcica, which is manifested by disturbed porphyrin metabolism, cutaneous lesions and hyperpigmentation. The authors estimated that 0.05-0.2 g/day were ingested. In children under 1 year of age, pink sore was observed as well as 95% mortality in these infants.

Follow-up studies conducted with patients 20-25 years after the onset of porphyria showed that a few patients still had active porphyria, whereas >50% exhibited hyperpigmentation scarring as well as other dermatologic, neurologic and skeletal features of hexachlorobenzene toxicity. Hexachlorobenzene residues were also found in the blood, fat or breast milk of some patients.

A correlation was found between hexachlorobenzene levels in blood and the number of years worked in a chlorinated solvents plant. The concentration of urinary uroporphyrins and coproporphyrins in workers ranged from 21-37 and 67-101 μg/ℓ, respectively, for the period between 1974 and 1977. An epidemiologic survey conducted with 86 residents in the vicinity of this chlorinated solvents plant showed elevated hexachlorobenzene residues in plasma. Higher levels of hexachlorobenzene residues were found in males than in females, but these were not associated with race or food consumption.

VII. MECHANISMS OF TOXICITY

Mechanism of Porphyria

Rizzardini and Smith (1982) clearly indicated that female rats are more susceptible to hexachlorobenzene-induced porphyria than are male rats, and that this difference in susceptibility is probably associated with the faster metabolism of hexachlorobenzene in females. They intubated male and female F344/N rats every other day for 103 days with 14 mg/kg (50 μmole/kg) hexachlorobenzene dissolved in arachis oil and monitored the rats for hexachlorobenzene metabolites and porphyrin levels. The results indicated that after 75 days of hexachlorobenzene treatment the excretion of urinary porphyrins increased rapidly in the females and after 103 days the females had urine and liver porphyrin levels 40- and 310-fold higher, respectively, than did the males. During this treatment period the females were found to excrete greater quantities of hexachlorobenzene metabolites, especially pentachlorothiophenol, than the males. Estrogen levels seem to play an important part in the increased susceptibility of females to hexachlorobenzene-induced porphyria. When both male and female rats were pretreated intraperitoneally with four doses of 20 μmole/kg of diethylstilboestrol dipropionate (an estrogenic drug), both sexes had stimulated excretion of hexachlorobenzene metabolites.

Graef et al. (1979) reported that hexachlorobenzene blocked the activity of rat hepatic 3-hydroxysteroid dehydrogenase leading to the accumulation of 5β-H-steroids, which are known inducers of porphyrin biosynthesis. Hexachlorobenzene-induced porphyria has also been reported to occur as a result of a deficiency in the uroporphyrinogen decarboxylation process that is catalyzed by porphyrinogen carboxylase. This enzyme is the only one in the heme pathway that exhibits a decrease in activity. The inhibition of porphyrinogen carboxylase in liver homogenates from female Wistar rats with severe porphyria induced by hexachlorobenzene was studied by Rios de Molina et al. (1980). Hexachlorobenzene had no effect on enzyme activity at 10^{-3} M, whereas pentachlorophenol caused a 90% inhibition at the same concentration. However, pentachlorophenol did not inhibit the enzyme at a concentration of 10^{-5} M. It was concluded that a concentration $>10^{-5}$ M of pentachlorophenol, possibly together with other hexachlorobenzene metabolites, was needed to cause enzyme inhibition.

A better understanding of hexachlorobenzene-induced porphyria was provided by Koss et al. (1983). These researchers administered every other day for 6 weeks, through stomach tube, 100 mg/kg hexachlorobenzene dissolved in olive oil to female Wistar rats and then observed the rats for an additional 18 months. The rats were evaluated during both the exposure period and the 18-month holding period for liver hexachlorobenzene levels, levels of liver porphyrins, and the activity of liver uroporphyrinogen decarboxylase. The results revealed a rapid increase in hexachlorobenzene liver levels which reached a plateau after 10 days of treatment and remained constant until exposure was terminated at 6 weeks. The levels of liver hexachlorobenzene then decreased over time with no valid biological halflife determinable. The liver porphyrin levels, however, started to rise slightly after 3 weeks of hexachlorobenzene exposure and reached a maximum liver porphyrin concentration ~7 months after the exposures had ceased (Table VII-1). The liver porphyrin levels decreased to a constant level ~14 months after ceasing hexachlorobenzene exposures. At 18 months after ceasing exposures, the treated rats liver porphyrin levels were still substantially

TABLE VII-1

Porphyrin Content and Uroporphyrinogen Decarboxylase Activity in the Liver Cytosol of Female Rats Pretreated with 100 mg/kg HCB Every Other Day for 6 Weeks[a]

Time After the End of Treatment	Porphyrin Content (nmol/6 mℓ cytosol)[b]	Enzyme Activity (pmol • mg^{-1} • min^{-1})[c]
1 day	14 ± 3[d]	ND[e]
7 months	133 ± 15	ND
14 months	9 ± 6	ND
18 months	8 ± 5	0.3 ± 0.2[d]
Controls	0.06 ± 0.04	0.5 ± 0.1

[a] Source: Koss et al., 1983

[b] 6 mℓ cytosol correspond with 1 g liver tissue

[c] pmol coproporphyrinogen I (determined as coproporphyrin) formed from uroporphyrinogen I in 1 min by 1 mg cytosol protein

[d] Mean (± SD) of three or four animals

[e] ND = Not detectable. The lower detection limit was determined at 0.02 pmol • mg^{-1} • min^{-1} coproporphyrin

HCB = Hexachlorobenzene

higher than the levels in control rats. The distribution pattern of the liver porphyrins was observed to be changed as early as after the second hexachlorobenzene administration. The observed changes were increases in liver uroporphyrin levels and decreases in liver protoporphyrin and coproporphyrin levels. The change in porphyrin patterns was traced to the decreased activity of uroporphyrinogen decarboxylase activity which was found to be not detectable at the end of the 6-week exposure period and the activity did not become detectable again until 18 months postexposure (see Table VII-1). These data led the investigators (Koss et al., 1983) to propose that there are four phases of hexachlorobenzene-induced porphyria:

> During the first phase an almost constant content of hexachlorobenzene and a gradual decrease of uroporphyrinogen decarboxylase activity is achieved. In the second phase a noticeable accumulation of porphyrins and a practically complete inhibition of decarboxylase activity are conspicuous. In the third phase, which occurs after hexachlorobenzene administration has been discontinued, a further accumulation of porphyrins and a continuing inhibition of uroporphyrinogen decarboxylase activity can be seen, even after extensive elimination of hexachlorobenzene. During the fourth phase a decrease in porphyrin content and a return of decarboxylase activity are clearly observable.

A possible reason for the continued inhibition of uroporphyrinogen decarboxylase activity, even after substantial elimination of hexachlorobenzene has occurred, was also discussed in this report. Koss et al. (1983) presented the scenario that once hexachlorobenzene had caused an inhibition of uroporphyrinogen decarboxylase activity and increased liver porphyrin levels that the accumulation of porphyrins could themselves maintain the inhibition of the enzyme activity.

Interactions

Certain chemicals have been shown to alter the toxicity and pharmacokinetics of hexachlorobenzene in mammals. Pentachlorophenol and iron increased the porphyrinogenic effect of hexachlorobenzene, whereas decachlorobiphenyl had no effect. Hexachlorobenzene pretreatment resulted in increased CCl_4 toxicity and altered immune responses in hexachlorobenzene-treated animals. In addition, hexachlorobenzene has been shown to induce hepatic xenobiotic metabolism and thus has the potential to alter the rate and extent of metabolism of other chemicals.

Debets et al. (1980b) studied the effect of pentachlorophenol (PCP) on hexachlorobenzene toxicity. Groups of female rats were fed diets containing 1000 μg hexachlorobenzene/g, 500 μg pentachlorophenol/g, or both chemicals in the same amounts, and a fourth group served as the control. Pentachlorophenol accelerated the onset of hexachlorobenzene-induced porphyria, as indicated by an increase in urinary excretion of uroporphyrin and a decrease of porphyrins with two and three carboxylic groups. This increase occurred ~3 weeks earlier in the hexachlorobenzene plus pentachlorophenol-treated animals than in hexachlorobenzene-treated animals.

Razzardini and Smith (1982) investigated diethylstilboestrol (DES) pretreatment on hexachlorobenzene metabolite excretion in young male and female F344/N rats. The rats were injected i.p. with four doses of DES dipropionate 20 μmoles/kg dissolved in arachis oil over a 24-day period and then given 14 mg/kg hexachlorobenzene by oral intubation for 7 days. The results indicated that the DES pretreatment stimulated the excretion, via urine and feces, in both males and females (Table VII-2).

Blekkenhorst et al. (1980) reported that the simultaneous i.m. administration of iron and hexachlorobenzene caused a marked potentiation of hexa-

TABLE VII-2

Analysis of the Excreta from Rats Administered Hexachlorobenzene After an Initial Treatment with Diethylstilboestrol[a,b]

Sex and Treatment	Pentachlorophenol	Tetrachlorobenzene-1,4-diol (nmole/24 hours/kg bw)	Pentachlorothiophenol
Urine			
Male + oil	151 ± 19	3 ± 1	23 ± 3
Male + DES	190 ± 22	17 ± 2[c]	158 ± 9[c]
Female + oil	174 ± 17	16 ± 2[d]	142 ± 12[e]
Female + DES	453 ± 105[f]	35 ± 9	176 ± 7[f]
Feces			
Male + oil	85 ± 15	Trace	74 ± 23
Male + DES	160 ± 23[f]	Trace	166 ± 33
Female + oil	116 ± 35	Trace	65 ± 4
Female + DES	279 ± 80	Trace	149 ± 13[c]

[a] Source: Razzardinia and Smith, 1982

[b] Male and female rats (52-54 and 71-73 days old, respectively) were given 20 μmole of DES dipropionate/kg dissolved in arachis oil (10 mg/mℓ) or oil alone by i.p. injection on days 1, 4, 14 and 24. From day 25 all rats were given 14 mg of hexachlorobenzene/kg by oral intubation daily for 7 days. After the last dose 24-hour samples of urine and feces were collected, hydrolyzed and analyzed. Results are means ± S.E.M. (n=4/group).

[c] Significance of differences from rats not given DES, $p<0.001$

[d] Significance of differences from males, $p<0.005$

[e] Significance of differences from males, $p<0.001$

[f] Significance of differences from rats not given DES, $p<0.05$

Total excretions of these metabolites were: male, 336±57; male + DES, 691±70 ($p<0.01$); female, 513±62; female + DES, 1092±175 ($p<0.025$) nmole/24 hours/kg chlorobenzene porphyrinogenic effect in rats. This was shown by a decrease in hepatic uroporphyrinogen decarboxylase activity and increased urinary and fecal porphyrin excretion. Conversely, simultaneous bleeding of hexachlorobenzene-treated rats diminished the porphyrinogenic effect of hexachlorobenzene.

Goldstein et al. (1978) studied the comparative toxicity of pure hexachlorobenzene (purity >99%) and technical hexachlorobenzene (purity 92%) which was known to contain 200 ppm of decachlorobiphenyl and 4 ppm of octachlorodibenzofuran, in female CD rats fed diets containing 0, 30, 100, 300 or 1000 μg hexachlorobenzene/g for up to 15 weeks. Neither grade contained other chlorinated dibenzofurans or dibenzo-p-dioxins. Both grades resulted in comparable effects (porphyria, cutaneous lesions, hyperexcitability, changes in liver enzymes and morphological liver changes) in treated rats, although the technical grade appeared to be slightly more potent than pure hexachlorobenzene in its effects on the pulmonary endothelium. The impurities did not appear to have a synergistic effect.

Kluwe et al. (1982) reported that pretreatment of male Sprague-Dawley rats with hexachlorobenzene resulted in increased CCl_4 toxicity. The rats received seven doses of hexachlorobenzene at 30 mg/kg in corn oil once every 72 hours followed by an i.p. injection of CCl_4 at 0.0, 0.03, 0.05, 0.25, 1.0 or 2.0 mℓ/kg in 4 mℓ/kg corn oil 24 hours after the last hexachlorobenzene treatment. Hexachlorobenzene pretreatment increased the CCl_4-induced acute growth retardation, renal tubular functional impairment, hepatocellular necrosis and further reduced the survival of the animals. Variable results were reported in a study on the effect of hexachlorobenzene pretreatment of male albino Sprague-Dawley rats on the *in vivo* biotransformation, residue deposition, and elimination of ^{14}C-aldrin, 1-naphthol, DDT, hexachlorobenzene or mirex (Clark et al., 1981a). There was no evidence of qualitative changes in the biotransformation of any test compound that could be attributed to hexachlorobenzene pretreatment. Analysis of residue deposition gave mixed results: less ^{14}C residues were found in rats fed diets containing hexachlorobenzene and then treated with ^{14}C-aldrin, more ^{14}C residues were found after ^{14}C-DDT or ^{14}C-mirex treatment, and no difference was evident after ^{14}C-hexachlorobenzene or ^{14}C-1-naphthol treatment. Hexachlorobenzene also potentiates the effects of stress on male Sprague-Dawley rats (Clark et al., 1981). Rats fed 250 ppm hexachlorobenzene resulted in an increased severe loss of body weight when placed into crowded cages and compared to the weight loss of crowded control rats. Crowded rats fed hexachlorobenzene had higher tissue residues of hexachlorobenzene and higher mortality than the non-crowded hexachlorobenzene-treated rats or the control rats.

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

The quantification of toxicological effects of a chemical consists of an assessment of the noncarcinogenic and carcinogenic effects. In the quantification of noncarcinogenic effects, an acceptable daily intake (ADI) is calculated. An adjusted acceptable daily intake (AADI) and health advisory (HA) values for the chemical are then calculated to define the appropriate drinking water concentrations to limit human exposure. For ingestion data, this approach is illustrated as follows:

$$\text{ADI} = \frac{\text{(NOAEL or LOAEL in mg/kg/day) (body weight in kg)}}{\text{Uncertainty/Safety Factor}} = \text{mg/day}$$

$$\text{AADI} = \frac{\text{ADI}}{\text{Drinking water volume in } \ell\text{/day}} = \text{mg}/\ell$$

where:

NOAEL = no-observed-adverse-effect level
LOAEL = lowest-observed-adverse-effect level
Body weight = 70 kg for adult or 10 kg for child
Drinking water volume = 2 ℓ/day for adults or 1 ℓ/day for children
Uncertainty/Safety Factor = 10, 100 or 1000

Utilizing these equations, the following drinking water concentrations are developed for noncarcinogenic effects:

1. A 1-day HA for 10 kg child
2. A 1-day HA for 70 kg adult
3. A 10-day HA for 10 kg child
4. A 10-day HA for 70 kg adult
5. A lifetime AADI for a 70 kg adult

The distinctions made between the HA calculations (items 1-4) are associated with the duration of anticipated exposure. Items 1 and 2 assume a single acute exposure to the chemical. Items 3 and 4 assume a limited period of exposure (possibly 1-2 weeks). The HA values will not be used in establishing a drinking water standard for the chemical. Rather, they will be used as informal scientific guidance to municipalities and other organizations when emergency spills or contamination situations occur. The AADI value (item 5) is intended to provide the scientific basis for establishing a drinking water standard based upon noncarcinogenic effects.

A NOAEL or LOAEL is determined from animal toxicity data or human effects data. For animal data, this level is divided by an uncertainty factor because there is no universally acceptable quantitative method to extrapolate from animals to humans. The possibility must be considered that humans are more sensitive to the toxic effects of chemicals than are animals. For human data, an uncertainty factor is also used to account for the heterogeneity of the human population in which persons exhibit differing sensitivities to toxic chemicals. An Office of Drinking Water (ODW) modification of the guidelines set forth by the National Academy of Sciences (NAS, 1977, 1980) are typically used in establishing uncertainty factors as follows:

- An uncertainty factor of 10 is used when good acute or chronic human exposure data are available and supported by acute or chronic toxicity data in other species.
- An uncertainty factor of 100 is used when good acute or chronic toxicity data identifying NOEL/NOAEL are available for one or more species, but human data are not available.
- An uncertainty factor of 1000 is used when limited or incomplete acute or chronic toxicity data are available or when only the acute or chronic toxicity data identify a LOAEL (but not NOEL/NOAEL) for one or more species are available.

The uncertainty factor used for a specific risk assessment is judgmental. Factors that cannot be incorporated in the NAS/ODW guidelines (NRC, 1977) for selection of an uncertainty factor, but must be considered, include the following: 1) the quality of the toxicology data, 2) the significance of the adverse effect, and 3) the existence of counterbalancing beneficial effects.

If toxicological evidence requires the chemical to be classified as a potential carcinogen, mathematical models are used to calculate the estimated excess cancer risks associated with the ingestion of the chemical by drinking water. The bioassay data used in these estimates are from animal experiments. In order to predict the risk for humans, these data must be converted to an equivalent human dose. This conversion includes correction for noncontinuous animal feeding, nonlifetime studies and for the difference in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average human body weight is 70 kg and that the average human consumes 2 ℓ of water per day. The multistage model is then fit to the equivalent human data to estimate the risk at low doses. The upper 95% confidence limit of this estimate is used. Excess cancer risks can also be estimated using other models such as the one-hit model, the Weibull model, the logit model and the probit model. There is no basis in the current understanding of the biological mechanisms involved in cancer to choose among these models. The estimates of low doses for these models can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of risk rate levels has an inherent uncertainty. This is because the tools of scientific measurement, by their very nature, involve both systematic and random error. In most cases, only studies using experimental animals have been performed. There is thus uncertainty when the data are extrapolated to humans. When developing risk rate levels, several other areas of uncertainty exist, such as 1) incomplete knowledge concerning the health effects of contaminants in drinking water, 2) the impact of test animal age, sex and species and the nature of target organ systems examined on the toxicity study results, and 3) the actual rate of exposure of internal targets in test animals or humans. Dose-response data are usually only available for high levels of exposure, not for the lower levels of exposure for which a standard is being set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

Existing Guidelines, Recommendations and Standards

Occupational. Workplace standards have not been established in the U.S. The USSR has established a TLV of 0.08 ppm (0.9 mg/m^3) (Verschueren, 1977). In 1978, NIOSH classified hexachlorobenzene (HCB) as a Group II pesticide and recommended criteria for standards for occupations in pesticide manufacturing and formulating. These standards rely on engineering controls, work practices and medical surveillance programs, rather than workplace air limits, to protect workers from the adverse effects of pesticide exposure in manufacturing and formulating. NIOSH specifically chose not to establish scientifically valid environmental (workplace air) limits for pesticides (except those already promulgated), because exposure via other routes, especially dermal, had proven to be of critical importance for many pesticides and because NIOSH believed that "immediate action" was needed to protect workers in pesticide manufacturing and formulating plants (NIOSH, 1978).

Food. USDA regulates the use of hexachlorobenzene as a seed treatment for the control of wheat bunt (smut) under the Federal Seed Act (7 CFR 201).

Water. The U.S. EPA (1980), in an Ambient Water Quality Criteria Document for Chlorinated Benzenes, determined that hexachlorobenzene is a suspected human carcinogen and since there is no recognized safe concentration for a human carcinogen, the recommended concentration of hexachlorobenzene in water for maximum protection of human health is zero. However, a zero level was thought to be unfeasible in some cases; therefore, water levels that may result in incremental increases in cancer risk of lifetime exposure were estimated at 10^{-5} (one additional case of cancer for every 100,000 people exposed), 10^{-6} and 10^{-7}. The corresponding recommended criteria were 7.2, 0.72 and 0.072 ng/ℓ, respectively, assuming humans consume 2 ℓ of water and 6.5 g of fish and shellfish per day. The criteria were calculated by using the hepatoma data on male hamsters in the Cabral et al. (1977) carcinogenicity study and the multistage model.

Using the tumor data from the Cabral et al. (1979) carcinogenicity study with Swiss mice and the multistage model, the NAS (1983) averaged cancer risks based on the male and female mouse data to obtain a 95% upper limit of cancer risk of 1.85×10^{-6} with lifetime daily consumption of 1 ℓ of water containing 1 μg of hexachlorobenzene. Earlier, the NAS (1980) used this method and the tumor data in the Cabral et al. (1977) carcinogenicity study with male and female hamsters to calculate a 95% upper limit of cancer risk of 2.9×10^{-5}. The NAS (1980) also calculated a 7-day suggested no adverse response level (SNARL) of 0.03 mg/ℓ for hexachlorobenzene.

Overview of Effects

The toxicity of long-term dietary exposure of humans to hexachlorobenzene was demonstrated by the epidemic of porphyria cutanea tarda (PCT) in Turkish citizens who accidentally consumed bread made from grain treated with hexachlorobenzene (Cam, 1963; Peters et al., 1966, 1982). The authors estimated that 0.05-0.2 g/day were ingested. In children under 1 year of age, pink sore was observed as well as 95% mortality. In addition to the PCT-associated symptoms of skin lesions, hypertrichosis and hyperpigmentation, the exposure caused neurotoxicity and liver damage. Follow-up studies reported PCT symptoms, reduced growth and arthritic changes in the appendages of children who were directly or indirectly (i.e., through breast milk) exposed. Studies in rats have demonstrated hexachlorobenzene's ability to increase the incidence of stillbirths, decrease fetal growth and decrease postnatal survival (Grant et al., 1977; Khera, 1974). A study in rats reported that administration of hexachlorobenzene during gestation increased significantly the number of fetuses with extra ribs. A study in mice found that hexachlorobenzene given on days 7-16 of gestation resulted in an increased incidence of fetal abnormalities when compared with controls (Courtney et al., 1976).

The acute oral toxicity of hexachlorobenzene has been found to be low, with LD_{50} values ranging from 1700-10,000 mg/kg (NAS, 1977; IARC, 1979; Sax, 1979). Subchronic oral toxicity studies with a number of mammalian species indicated statistically significant increases in liver and kidney weights in hexachlorobenzene-treated animals (Kuiper-Goodman et al., 1977; Boger et al., 1979; Koss et al., 1978b; Sundlof et al., 1981; Shirai et al., 1978). Studies have shown increases in other organs as well (Elissalde and Clark, 1979; Koss et al., 1978b). The livers from hexachlorobenzene-exposed animals have shown histologic changes such as irregular shaped and moderately enlarged liver mitochondria and increases in the size of the centrilobular hepatocytes (Kuiper-Goodman et al., 1977; Boger et al., 1979; Lambrecht et al., 1982). Chronic oral toxicity studies revealed the same type of effects seen in the subchronic studies plus hexachlorobenzene-associated life-shortening and various hepatic and renal pathologies (Cabral et al., 1977, 1979; Smith and Cabral, 1980; Lambrecht et al., 1983a,b; Arnold et al., 1983). These subchronic and chronic effects were usually dose-related. Multiple alopecia and scabbing, together with neurologic effects, have also been observed in rats, mice and dogs (Headley et al., 1981; Sundlof et al., 1981). A dose-related histopathologic change in the ovaries of monkeys has also been reported (Iatropoulos et al., 1976; Knauf and Hobson, 1979).

Increased porphyrin levels in the liver and in urine have been reported for all species studied (Kuiper-Goodman et al., 1977; Koss et al., 1978, 1983; Smith et al., 1980; Gralla et al., 1977; Rizzardini and Smith, 1982) except for the dog, which does not exhibit increased porphyrin levels (Gralla et al., 1977). Hexachlorobenzene was found to cause the accumulation of 5ß-H-steroids which induce porphyrin biosynthesis (Graef et al., 1979), and to inhibit uroporphyrinogen decarboxylases (Graef et al., 1979; Koss et al., 1983). The inhibition of uroporphyrinogen decarboxylases appears to be due to pentachlorophenol, a hexachlorobenzene metabolite (Rio de Molina et al., 1980). There is evidence that females are more susceptible to hexachlorobenzene-induced porphyria than are males, which may be related to the female estrogen levels and greater hexachlorobenzene metabolism (Rizzardini and Smith, 1982). Hexachlorobenzene was reported to produce a mixed-type induction of cytochromes resembling that produced by a combination of phenobarbital (P-450) and 3,4-benzpyrene (P-448) (Goldstein et al., 1982; Debets et al., 1980a). In addition, the activities of hepatic microsomal enzymes were found to be induced by hexachlorobenzene (Ariyoshi et al., 1974; Mehendale et al., 1975; Carlson and Tardiff, 1976; Chadwick et al., 1977; Carlson, 1978, 1980; Carlson et al., 1979).

Hexachlorobenzene has been shown to produce various types of tumors in animals. Lifetime dietary administration of hexachlorobenzene to hamsters, rats and mice increased the incidence of thyroid tumors in hamsters (Cabral et al., 1977), liver tumors in hamsters (Cabral et al., 1977), mice (Cabral et al., 1979) and rats (Smith and Cabral, 1980; Lambrecht, 1983; Arnold, 1984), kidney tumors in rats (Lambrecht, 1983) and adrenal tumors in rats (Arnold, 1983; Peters et al., 1983).

Quantification of Noncarcinogenic Effects

Table VIII-1 presents a summary of the toxicity studies on hexachlorobenzene which were considered for calculation of the 1- and 10-day Health Advisories (HAs) and the lifetime adjusted acceptable daily intake (AADI).

TABLE VIII-1

Summary of Toxicity Studies on Hexachlorobenzene

Species	Route	Dose*	Duration	Effects	Reference
Rat (females)	oral	100 mg/kg every other day	up to 43 days	Suggested covalent binding of hexachlorobenzene metabolites to cytosolic proteins	Koss et al., 1980a
Rat	oral (diet)	0.5 mg/kg/day	15 weeks exposed and held to 48 weeks	Transient increases in liver porphyrin levels in females after termination of exposure	Kuiper-Goodman et al., 1977
		2.0 mg/kg/day	15 weeks exposed and held to 48 weeks	Increases in liver porphyrin levels in females after termination of exposure, increased size of centrilobular hepatocytes	
		8.0 mg/kg/day	15 weeks exposed and held to 48 weeks	Increased liver weights, increased liver, kidney and spleen porphyrin levels in females (porphyria), centrilobular liver lesions especially in females at 48 weeks	
		32.0 mg/kg/day	15 weeks exposed and held to 48 weeks	Increased mortality in females, intension tremors in males and females and ataxia in a few females, increased liver, kidney and spleen weights, increased liver, kidney and spleen porphyrin levels in females (porphyria), centrilobular liver lesions and splenomegaly	
Rat (females)	oral (gavage)	50 mg/kg every other day	15 weeks	Increased liver, kidney, spleen and adrenal weights, porphyria (increased liver porphyrin levels and increased excretion of porphyrins and precursors), tremors, hair loss and skin lesions	Koss et al., 1978b
Rats (females)	oral (gavage)	0.5 mg/kg twice weekly	29 weeks	Increase in relative liver weight	Böger et al., 1979
		2.0 mg/kg twice weekly	29 weeks	Increase in relative liver weight, moderately enlarged hepatocytes	
		8.0 mg/kg twice weekly	29 weeks	Porphyria, markedly enlarged hepatocytes, increase in relative liver weight	
		32.0 mg/kg twice weekly	29 weeks	Porphyria, markedly enlarged hepatocytes, increase in liver weights	
Rat (females)	oral (diet)	100 mg/kg diet	98 days	Porphyria (increased liver lobe porphyrins), decreased activity of uroporphyrinogen decarboxylase	Smith et al., 1980

TABLE VIII-1 (cont.)

Species	Route	Dose*	Duration	Effects	Reference
Rat	oral (diet and nursing)	50 mg/kg diet	gestation until 5 weeks of age	Depressed resistance to *L. monocytogenes* and *T. spiralis*, enhanced thymus-dependent antibody response	Vos et al., 1979b
		150 mg/kg diet	gestation until 5 weeks of age	Increased serum IgM and IgG, depressed resistance to *L. monocytogenes* and *T. spiralis*, enhanced thymus-dependent antibody response, increased liver and adrenal weights	
Rat	oral (diet)	500, 1000 or 2000 mg/kg diet	3 weeks	Dose-related increases in relative spleen, lymph nodes, liver, adrenals, thyroid, testes and kidney weights, dose-related increase in serum IgM levels, no change in serum IgG levels, dose-related pathological changes in liver, lymph nodes and spleen	Vos et al., 1979a
Rat	oral (diet)	2000 mg/kg diet	10 weeks	Porphyria found microscopically at 5 weeks and grossly at 10 weeks using fluorescence	Gralla et al., 1977
Rat (male)	oral (diet)	2000 mg/kg diet	100 days	Elevated hepatic enzymes by 1 week and increased urinary porphyrin and ALA levels (porphyria) as early as 40 days	Lissner et al., 1975
Rat (female)	oral (diet)	3000 mg/kg diet	11 weeks	Decreased uroporphyrinogen decarboxylase activity and porphyria after 4 weeks	Elder et al., 1976
Rat (female)	oral (gavage)	50, 100 or 200 mg/kg	120 days	Dose- and time-dependent increase in liver and urine porphyrins (porphyria)	Carlson, 1977
Rat	oral (gavage)	14 mg/kg every other day	103 days	Porphyria in treated females, susceptibility of females to porphyria may be related to estrogen levels	Rizzardini and Smith, 1982
Rat (females)	oral (gavage)	100 mg/kg every other day	6 weeks exposed and held for additional 18 months	Porphyria (liver uroporphyrin levels peaked 7 months postexposure and levels had not returned to normal by 18 months), decreased liver protoporphyrin and coproporphyrin levels, inhibition of uroporphyrinogen decarboxylase activity until 18 months postexposure	Koss et al., 1983
Rat (females)	oral (diet)	6-8 mg/kg/day	75-90 weeks	Decline in body weights, porphyria, enlarged livers and liver tumors	Smith and Cabral, 1980
Rat	oral (diet)	75 mg/kg diet (4-5 mg/kg/day) 150 mg/kg diet (8-9.5 mg/kg/day)	up to 2 years	Porphyria, time-related appearance of severe hepatic and renal pathologies, after 1 year increases in hepatomas, hepatocarcinomas, bile duct adenomas, renal adenomas and renal carcinomas	Lambrecht et al., 1983a,b

TABLE VIII-1 (cont.)

Species	Route	Dose*	Duration	Effects	Reference
Rat	oral (diet)	0.32, 1.6, 8.0 or 40 mg/kg diet	~130 days	Hematological changes at all dose levels in males, increases in liver and heart weights in males at 8.0 and 40 ppm diets, no treatment-related effects observed in bred females	Arnold et al., 1985
	oral (diet and nursing)	0.32 or 1.6 mg/kg diet	gestation through lifetime (130 weeks)	Glycogen depletion in 1.6 mg/kg males; no effects reported at 0.32 mg/kg	
		8.0 mg/kg diet	gestation through lifetime (130 weeks)	Increase in liver pathologies	
		40 mg/kg diet	gestation through lifetime (130 weeks)	Increased mortality as pups, increase in liver and kidney pathologies, increase in adrenal pheochromocytomas in females and parathyroid tumors in males	
Rat	oral (diet)	10 or 20 mg/kg diet	F_0 to F_4 generations	No effects reported	Grant et al., 1977
		40 mg/kg diet	F_0 to F_4 generations	Increases in liver weights and aniline hydroxylase activity	
		80 mg/kg diet	F_0 to F_4 generations	Decreased body weights, F_3 and F_4 generations had decreased lactation index and postnatal viability and increased stillbirths	
		160 mg/kg diet	F_0 to F_4 generations	Increased mortality and decreased lactation index starting in F_1 generation	
		320 and 640 mg/kg diet	F_0 to F_4 generations	20 and 50% mortality in F_0 320 and 640 mg/kg groups, respectively, greatly reduced fertility index and litter size and increase in stillbirths, viability index zero in F_1	
Rat	oral (diet)	60, 80, 100, 120 or 140 mg/kg diet	F_0 to F_{1a} and F_{1b} generations	Increased mortality in all groups at 21 days, 21-day LD_{50} values for pups were 100 and 140 mg/kg for F_{1a} and F_{1b} generations, respectively	Kitchin et al., 1982
Rat	oral (diet)	0 or 80 mg/kg diet	gestation and nursing or cross nursed with controls	Nursing exposure produced greater effects than did gestational exposure, effects noted were: smaller brains, hearts, kidneys and spleens, increased liver weights	Mendoza et al., 1978
Rat	oral (diet)	80 mg/kg diet	2 weeks prior to mating to 35-36 days after weaning	Increased porphyrin levels and decreased liver esterase activity in dams, no changes in gestation indices or neonatal survival	Mendoza et al., 1979

TABLE VIII-1 (cont.)

Species	Route	Dose*	Duration	Effects	Reference
Rat	oral (gavage)	10, 20, 40, 60, 80 or 120 mg/kg	days 6-21 of gestation	Maternal toxicity (weight loss, tremors and convulsions) and reduced fetal weights at 120 and 80 mg/kg maternal doses, dose-related increase in incidence of unilateral and bilateral 14th rib, sternal defects were also noted in one experiment	Khera, 1974
Mouse	oral (diet)	2.5, 25 or 250 mg/kg diet	21 days	Dose-related increase in liver and decrease in prostate and seminal vesicle weights, dose-related alterations in testosterone metabolism, altered hepatic enzyme levels	Elissalde and Clark, 1979
Mouse (male)	oral (diet)	10 mg/kg diet (8.4 (mg/mouse/24 weeks) or 50 mg/kg diet (35.3 mg/mouse/ 24 weeks)	24 weeks	Dose-related reduction in weight gain, no tumor pathology observed	Shirai et al., 1978
Mouse (male)	oral (diet)	167 mg/kg diet	3-6 weeks	Impairment in host resistance as measured by increased sensitivity to *S. typhosa* and *P. bergheri*, and decrease in IgA levels	Loose et al., 1978a,b
Mouse	oral (diet)	6, 12, 24 and 36* mg/kg/day	101-120 weeks *(15 weeks exposed held until 120 weeks)	Reduced growth rate at all dose levels, shortened lifespan associated with tremors and convulsions in 24 and 36 mg/kg/day groups, dose-dependent increase in liver-cell tumors in the 12, 24 and 36 mg/kg/day dose groups	Cabral et al., 1979
Mouse	oral (gavage)	100 mg/kg/day to pregnant mice	days 7-16 of gestation	Increased maternal livers and decreased fetal body weights, increased incidence of abnormal fetuses per litter observed	Courtney et al., 1976
Hamster	oral (diet)	200 or 400 mg/kg diet	90 days	Precirrhotic and cirrhotic hepatic lesions, bile-duct hyperplasias and hepatomas	Lambrecht et al., 1982
Hamster	oral (diet)	4, 8 or 16 mg/kg/day	lifespan	Shortened lifespan in 16 mg/kg/day group, increase in hepatomas at all dose levels, increase in liver haemangioendothelioma in males and females and an increase in thyroid alveolar adenomas in males in 16 mg/kg/day group	Cabral et al., 1977
Cats (breeding females)	oral (diet)	3 or 8.7 mg/day/cat	142 days	Weight loss and increased disease susceptibility in bred females, dose-related decrease in litter size and survival of offspring, hepatomegaly in offspring, all with the 8.7 mg/cat/day dose.	Hansen et al., 1979

TABLE VIII-1 (cont.)

Species	Route	Dose*	Duration	Effects	Reference
Minks	oral (diet)	1 or 5 mg/kg diet	during gestation until 17 weeks of age	Dose-related increase in offspring mortality, induction of hepatic MFO enzymes in exposed offspring	Rush et al., 1983
Dog (female)	oral (capsule)	50 or 150 mg/kg/day	21 days	Liver and hepatocyte enlargement, dose-induced electroencephalogram dysrhythmias	Sundlof et al., 1981
Dog	oral (capsule)	1, 10, 100 or 1000 mg/day/dog	1 year	Increase in mortality, neutrophilia, and anorexia in the 100 and 1000 mg dose groups, dose-related nodular hyperplasia of gastric lymphoid tissue in all treated animals	Gralla et al., 1977
Monkey (female)	oral (gavage)	8, 32, 64 or 128 mg/kg/day	60 days	Dose-related pathology in liver, kidney, ovaries and thymus	Iatropoulus et al., 1976
Monkey	oral (nursing)	7.51-186 ppm milk	60 days	2 of 3 infants died as a result of exposures	Bailey et al., 1980

*mg/kg doses are per kg of body weight unless shown as per kg of diet.

1-Day HA. Available evidence for the acute toxicity of hexachlorobenzene is considered to be insufficient for calculation of a 1-day HA. There are acute oral LD_{50} studies with hexachlorobenzene which indicate low acute toxicity, as LD_{50} values ≥1700 mg/kg, for this material in rats, rabbits, cats and mice. However, these studies do not provide an assessment of systemic toxicity which can be applied to a 1-day HA calculation. Conversely, the Kuiper-Goodman et al. (1977) study, which is the basis for the 10-day HA herein, gives a dose-response with effect and no-effect levels for systemic toxicity in male and female rats given hexachlorobenzene in the diet. The subchronic dosing pattern is a limitation in using the Kuiper-Goodman et al. (1977) study specifically for a 1-day HA calculation. None- theless, administration of hexachlorobenzene in the diet would more closely approximate exposure patterns with drinking water than bolus treatment via gavage or capsules, which was the method of treatment in the acute as well as several subchronic toxicity studies with hexachlorobenzene. Therefore, the 10-day HA is also proposed for use as a 1-day HA.

10-Day HA. The 15-week subchronic feeding study by Kuiper-Goodman et al. (1977) is selected for the derivation of the 10-day HA. The doses used in this study are on a mg hexachlorobenzene/kg bw basis which is a preferred dose unit for HA calculations, and the data given in the study report show dose responses with effect and no-effect levels for the effects observed. In the study by Arnold et al. (1983), F_0 males used as breeders for the F_1 generation had statistically significant increases in liver-to-body weight ratios and heart weights after exposure to 8 and 40 ppm hexachlorobenzene in the diet for 130 days. The study by Arnold et al. (1983) could also be proposed for calculation of a 10-day HA, but the organ weight and body weight data were not reported to show the strength of the effects and the dose response, and the selection of the exposure levels on a ppm basis could result in a less precise estimate of dose on a mg/kg basis. If the assumption that a young rat can consume a daily amount of food equal to 10% of its body weight (Mitruka et al., 1976) is applied, then the 1.6, 8 and 40 ppm dietary hexachlorobenzene levels in the Arnold et al. (1983) study would be equivalent to 0.16, 0.8 and 4 mg/kg/day, respectively. On this estimated mg/kg basis, the dose response in the Arnold et al. (1983) study appears roughly consistent with that in the Kuiper-Goodman et al. (1977) study where 0.5 and 2 mg/kg/day were no-effect and lowest-effect levels, respectively.

Rush et al. (1983) observed a substantial reduction in the survival of mink offspring from mothers exposed to dietary levels of 1 and 5 ppm hexachlorobenzene from about 6 weeks before mating until weaning of offspring. Assuming a daily food consumption of 155 g and a body weight of 0.87 kg for female minks (Bleavins and Aulerich, 1981), 1 and 5 ppm dose levels of hexachlorobenzene would be equivalent to 0.155 and 0.775 mg/kg/day, respectively. Thus, the mink is quite sensitive to hexachlorobenzene effects on reproduction. However, this study is not proposed for use in the 10-day HA or AADI calculations in that the mink is a species which has not been extensively investigated as an animal model for toxicity testing. Furthermore, a 10-day exposure would be considerably shorter than the periods of gestation and lactation in humans that would correspond to the periods when mink offspring were exposed to hexachlorobenzene until weaning; the minks gastrointestinal physiology is different from that of humans; and no adverse systemic toxicity effects were found in mink offspring that were given hexachlorobenzene until sacrifice at 17 weeks of age.

In the Kuiper-Goodman et al. (1977) study, groups of 70 male and 70 female Charles River (COBS) rats were fed diets providing 0, 0.5, 2.0, 8.0 or 32.0 mg/kg bw/day of hexachlorobenzene, dissolved in corn oil, for as long as 15 weeks. Female rats were found to be more susceptible to hexachlorobenzene, as indicated by all parameters studied, and an "apparent" NOEL of 0.5 mg/kg/day was concluded by the authors. Increased liver porphyrin levels in females and increases in the size of centrilobular hepatocytes along with the depletion of hepatocellular marker enzymes were noted with higher doses. With the two highest doses, there were increased liver-to-body weight ratios in males and females and increased porphyrin levels in the kidney and spleen. Exposure to the highest dose resulted in decreased survival in females, spleenomegaly in females, increases in spleen-to-body weight and kidney-to-body weight ratios in males and females, intension tremors in males and females, ataxia in females, and decreased body weight in males. Similar effects in liver were reported by Boger et al. (1979) after treating groups of 36 female Wistar rats twice weekly for 29 weeks with oral doses of 0.5, 2.0, 8.0 and 32.0 mg hexachlorobenzene/kg bw in olive oil.

The 10-day HA is calculated using the NOEL of 0.5 mg/kg bw/day reported by Kuiper-Goodman et al. (1977), a 100-fold uncertainty factor which represents two 10-fold factors to account for both intra- and interspecies variability to the toxicity of chemicals when specific data are lacking, 70 kg as the adult body weight and 10 kg as the child body weight, and an assumed daily intake of 2 ℓ of water by an adult and 1 ℓ of water by a child.

For an adult:

$$\text{10-day HA} = (0.5 \text{ mg/kg bw/day} \times 70 \text{ kg}) \div (100 \times 2) = 0.175 \text{ mg/}\ell$$

This HA is equivalent to 0.35 mg/day or 0.005 mg/kg/day.

For a child:

$$\text{10-day HA} = (0.5 \text{ mg/kg bw/day} \times 10 \text{ kg}) \div (100 \times 1) = 0.050 \text{ mg/}\ell$$

This HA is equivalent to 0.050 mg/day or 0.005 mg/kg bw/day.

Using this formula and the estimated 0.16 mg/kg bw/day NOEL in the Arnold et al. (1983) study, the 10-day HA for a 70 kg adult and a 10 kg child would be 0.056 and 0.016 mg/ℓ, respectively.

Lifetime AADI. The derivation of the lifetime AADI is based on a 130-week study of Arnold et al. (1983). This study involved feeding male and female Sprague-Dawley rats, the F_0 generation, diets containing 0, 0.32, 1.6, 8.0 or 40 ppm of hexachlorobenzene (analytical grade) for 90 days before mating and until 21 days parturition (at weaning).

The number of offspring (F_1 generation) from these matings were reduced to 50 males and 50 females per dose group at 28 days of age and fed their respective parents' diets. Thus, the F_1 animals were exposed to hexachlorobenzene and metabolites in utero, from maternal nursing and from their diets for the remainder of their lifetime (130 weeks). No hexachlorobenzene-induced effects were reported in the 0.32 ppm hexachlorobenzene F_1 group, indicating this level is a NOEL. Although a significant ($p<0.05$) increase in the incidence of periportal glycogen depletion was found in F_1 male rats fed 1.6 ppm hexachlorobenzene, the 1.6 ppm level of hexachlorobenzene is also concluded to be a NOEL in that this result was not evident in other treated groups of male rats. The 8.0 ppm hexachlorobenzene F_1 groups were reported to have an increase ($p<0.05$) in hepatic centrilobular basophilic chromogenesis. The 40 ppm hexachlorobenzene F_1 groups were reported with increases ($p<0.05$) in pup mortality, hepatic centrilobular basophilic chromogenesis, peribiliary lymphocytosis and fibrosis, severe chronic nephrosis in males, adrenal pheochromocytomas in females and parathyroid tumors in males.

A lifetime AADI for hexachlorobenzene is calculated using a 100-fold uncertainty factor which represents two 10-fold factors to account for both the intra- and interspecies variability to the toxicity of the chemical when specific data are lacking. It is difficult to estimate lifetime doses on a mg hexachlorobenzene/kg bw basis in this study because of the initial exposure of the animals to hexachlorobenzene and its metabolites _in utero_ and during lactation. However, in an attempt to estimate the lifetime hexachlorobenzene doses on a mg/kg bw basis, the 1.6 mg/kg hexachlorobenzene diet level, interpreted from this study as the highest NOEL level, was converted to a daily intake dose of 0.08 mg/kg bw/day by averaging the dosage data provided by Arnold (1984). A 70 kg adult body weight and an assumed intake of 2 ℓ of water per day are used in the calculation.

For an adult:

$$\text{Lifetime AADI} = (0.08 \text{ mg/kg bw/day} \times 70) \div (100 \times 2) = 0.028 \text{ mg/}\ell$$

This AADI is equivalent to 0.056 mg/day or 0.0008 mg/kg bw/day.

Carcinogenic Effects

This quantitative section deals with estimation of the unit risk for hexachlorobenzene as a potential carcinogen in water, and with the potency of hexachlorobenzene relative to other carcinogens that have been evaluated by the U.S. EPA Carcinogen Assessment Group (CAG). The unit risk for a water pollutant is defined as the lifetime cancer risk to humans from daily exposure to a concentration of 1 μg/ℓ in water by ingestion. The U.S. EPA CAG has prepared the rationale and the calculation for the unit risk estimate presented herein for the U.S. EPA (1984) Health Assessment Document for Chlorinated Benzenes.

The unit risk estimate for hexachlorobenzene represents an extrapolation below the dose range of experimental data. There is currently no solid scientific basis for any mathematical extrapolation model that relates exposure to cancer risk at the extremely low concentrations, including the unit concentration given above, that must be considered when evaluating environmental hazards. For practical reasons, the correspondingly low levels of risk cannot be measured directly either by animal experiments or by epidemiologic study. Therefore, low dose extrapolation must be based on current understanding of the mechanisms of carcinogenesis. At the present time the dominant view of the carcinogenic process involves the concept that most cancer-causing agents also cause irreversible damage to DNA. This position is based in part on the fact that a very large proportion of agents that cause cancer are also mutagenic. There is reason to expect that the quantal response that is characteristic of mutagenesis is associated with a linear (at low doses) non-threshold dose-response relationship. Indeed, there is substantial evidence from mutagenicity studies with both ionizing radiation and a wide variety of chemicals that this type of dose-response model is the appropriate one to use. This is particularly true at the lower end of the dose-response curve; at high doses there can be an upward curvature, probably reflecting the effects of multistage processes on the mutagenic response. The linear non-threshold dose-response relationship is also consistent with the relatively few epidemiologic studies of cancer responses to specific agents that contain enough information to make the evaluation possible (e.g., radiation-induced leukemia, breast and thyroid cancer, skin cancer induced by arsenic in drinking water, liver cancer induced by aflatoxins in the diet). Some supporting evidence also exists from animal experiments (e.g., the initiation stage of the two-stage carcinogenesis model in rat liver and mouse skin).

Because its scientific basis, although limited, is the best of any of the current mathematical extrapolation models, the non-threshold model, which is linear at low doses, has been adopted as the primary basis for risk extrapolation to low levels of the dose-response relationship. The risk estimates made with such a model should be regarded as conservative, representing the most plausible upper limit for the risk (i.e., the true risk is not likely to be higher than the estimate, but it could be lower).

For several reasons, the unit risk estimate based on animal bioassays is only an approximate indication of the absolute risk in populations exposed to known carcinogen concentrations. First, there are important species differences in uptake, metabolism and organ distribution of carcinogens, as well as species differences in target site susceptibility, immunological responses, hormone function, dietary factors and disease. Second, the concept of equivalent doses for humans compared to animals on a mg/surface area basis is virtually without experimental verification in regard to carcinogenic response. Third and finally, human populations are variable with respect to genetic constitution and diet, living environment, activity patterns and other cultural factors.

The unit risk estimate can give a rough indication of the relative potency of a given agent as compared with other carcinogens. Such estimates are, of course, more reliable when the comparisons are based on studies in which the test species, strain, sex and routes of exposure are similar.

The quantitative aspect of carcinogen risk assessment is addressed here because of its possible value in the regulatory decision-making process, e.g., in setting regulatory priorities, evaluating the adequacy of technology-based controls, etc. However, the imprecision of presently available technology for estimating cancer risks to humans at low levels of exposure should be recognized. At best, the linear extrapolation model used here provides a rough but plausible estimate of the upper limit of risk -- that is, with this model it is not likely that the true risk would be much more than the estimated risk, but it could be considerably lower. The risk estimates presented in subsequent sections should not be regarded, therefore, as accurate representations of the true cancer risks even when the exposures involved are accurately defined. The estimates presented may, however, be factored into regulatory decisions to the extent that the concept of upper-risk limits is found to be useful.

Procedures for the Determination of Unit Risk.

Low Dose Extrapolation Model -- The mathematical formulation chosen to describe the linear non-threshold dose-response relationship at low doses is the linearized multistage model (Crump and Watson, 1979). This model employs enough arbitrary constants to be able to fit almost any monotonically increasing dose-response data, and it incorporates a procedure for estimating the largest possible linear slope (in the 95% confidence limit sense) at low extrapolated doses that is consistent with the data at all dose levels of the experiment.

Let P(d) represent the lifetime risk (probability) of cancer at dose d. The multistage model has the form:

$$P(d) = 1 - \exp [-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)]$$

where

$$q_i \geq 0, \text{ and } i = 0, 1, 2, \ldots, k$$

Equivalently,

$$P_t(d) = 1 - \exp [-(q_1 d + q_2 d^2 + \ldots q_k d^k)]$$

where

$$P_t(d) = \frac{P(d) - P(0)}{1 - P(0)}$$

is the extra risk over background rate at dose d.

The point estimate of the coefficients q_i, i = 0, 1, 2, ..., k, and consequently, the extra risk function, $P_t(d)$, at any given dose d, is calculated by maximizing the likelihood function of the data.

The point estimate and the 95% upper confidence limit of the extra risk, $P_t(d)$, are calculated by using the GLOBAL79 computer program developed by Crump and Watson (1979). At low doses, upper 95% confidence limits on the extra risk and lower 95% confidence limits on the dose producing a given risk are determined from a 95% upper confidence limit, q_1*, on parameter q_1. Whenever $q_1 > 0$, at low doses the extra risk $P_t(d)$ has approximately the form $P_t(d) = q_1^* \times d$. Therefore, $q_1^* \times d$ is a 95% upper confidence limit on the extra risk and R/q_1^* is a 95% lower confidence limit on the dose, producing an extra risk of R. Let L_0 be the maximum value of the log-likelihood function. The upper-limit q_1* is calculated by increasing q_1 to a value q_1* such that when the log-likelihood is remaximized subject to this fixed value q_1* for the linear coefficient, the resulting maximum value of the log-likelihood L_1 satisfies the equation

$$2\ (L_0 - L_1) = 2.70554$$

where 2.70554 is the cumulative 90% point of the chi-square distribution with one degree of freedom, which corresponds to a 95% upper-limit (one-sided). This approach of computing the upper confidence limit for the extra risk $P_t(d)$ is an improvement on previous models. The upper confidence limit for the extra risk calculated at low doses is always linear. This is conceptually consistent with the linear non-threshold concept discussed earlier. The slope, q_1*, is taken as an upper-bound of the potency of the chemical in inducing cancer at low doses. [In the section calculating the risk estimates, $P_t(d)$ will be abbreviated as P.]

In fitting the dose-response model, the number of terms in the polynomial is chosen equal to (h-1), where h is the number of dose groups in the experiment, including the control group.

Whenever the multistage model does not fit the data sufficiently, data at the highest dose is deleted and the model is refitted to the rest of the data. This is continued until an acceptable fit to the data is obtained. To determine whether or not a fit is acceptable, the chi-square statistic:

$$X^2 = \sum_{i=1}^{h} \frac{(X_i - N_iP_i)^2}{N_iP_i\,(1-P_i)}$$

is calculated where N_i is the number of animals in the i^{th} dose group, X_i is the number of animals in the i^{th} dose group with a tumor response, P_i is the probability of a response in the i^{th} dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups. The fit is determined to be unacceptable whenever X^2 is larger than the cumulative 99% point of the chi-square distribution with f degrees of freedom, where f equals the number of dose groups minus the number of non-zero multistage coefficients.

Selection of Data -- For some chemicals, several studies in different animal species, strains and sexes, each run at several doses and different routes of exposure, are available. A choice must be made as to which of the data sets from several studies to use in the model. It may also be appropriate to correct for metabolism differences between species and for absorption factors via different routes of administration. The procedures used in evaluating these data are consistent with the approach of making a maximum-likely risk estimate. They are as follows:

1. The tumor incidence data are separated according to organ sites or tumor types. The set of data (i.e., dose and tumor incidence) used in the model is the set where the incidence is statistically significantly higher than the control for at least one test dose level and/or where the tumor incidence rate shows a statistically significant trend with respect to dose level. The data set that gives the highest estimate of the lifetime carcinogenic risk, q_1^*, is selected in most cases. However, efforts are made to exclude data sets that produce spuriously high risk estimates because of a small number of animals. That is, if two sets of data show a similar dose-response relationship, and one has a very small sample size, the set of data having the larger sample size is selected for calculating the carcinogenic potency.

2. If there are two or more data sets of comparable size that are identical with respect to species, strain, sex and tumor sites, the geometric mean of q_1^*, estimated from each of these data sets, is used for risk assessment. The geometric mean of numbers A_1, A_2, ..., A_m is defined as

$$(A_1 \times A_2 \times \ldots \times A_m)^{1/m}.$$

3. If two or more significant tumor sites are observed in the same study, and if the data are available, the number of animals with at least one of the specific tumor sites under consideration is used as incidence data in the model.

Calculation of Human Equivalent Dosages -- Following the suggestion of Mantel and Schneiderman (1975), it is assumed that mg/surface area/day is an equivalent dose between species. Since, to a close approximation, the surface area is proportional to the two-thirds power of the weight, the exposure in mg/day$^{2/3}$ of the weight is also considered to be equivalent exposure. In an animal experiment, this equivalent dose is computed in the following manner.

Let

L_e = duration of experiment

l_e = duration of exposure

m = average dose per day in mg during administration of the agent (i.e., during l_e), and

W = average weight of the experimental animal

Then, the lifetime exposure is

$$d = \frac{l_e \times m}{L_e \times W^{2/3}}$$

Oral. Often exposures are not given in units of mg/day, and it becomes necessary to convert the given exposures into mg/day. Similarly, in drinking water studies, exposure is expressed as ppm in the water. For example, in most feeding studies exposure is given in terms of ppm in the diet. In these cases, the exposure in mg/day is

$$m = \text{ppm} \times F \times r$$

where ppm is parts per million of the carcinogenic agent in the diet or water, F is the weight of the food or water consumed per day in kg, and r is the absorption fraction. In the absence of any data to the contrary, r is assumed to be equal to one. For a uniform diet, the weight of the food consumed is proportional to the calories required, which in turn is proportional to the surface area, or two-thirds power of the weight. Water demands are also assumed to be proportional to the surface area, so that

$$m \propto ppm \times W^{2/3} \times r$$

or

$$\frac{m}{rW^{2/3}} \propto ppm.$$

As a result, ppm in the diet or water is often assumed to be an equivalent exposure between species. However, this is not justified in dose extrapolation of laboratory animals to humans since the ratio of calories to food weight is very different in the diet of man as compared to laboratory animals, primarily due to differences in the moisture content of the foods eaten. For the same reason, the amount of drinking water required by each species also differs. It is therefore necessary to use an empirically-derived factor, f = F/W, which is the fraction of an organism's body weight that is consumed per day as food, expressed as follows:

		Fraction of Body Weight Consumed as	
Species	W	f_{food}	f_{water}
Man	70	0.028	0.029
Rats	0.35	0.05	0.078
Mice	0.03	0.13	0.17

Thus, when the exposure is given as a certain dietary or water concentration in ppm, the exposure in mg/$W^{2/3}$ is

$$\frac{m}{rW^{2/3}} = \frac{\text{ppm} \times F}{W^{2/3}} = \frac{\text{ppm} \times f \times W}{W^{2/3}} = \text{ppm} \times f \times W^{1/3}$$

When exposure is given in terms of mg/kg/day = $m/Wr = s$, the conversion is simply

$$\frac{m}{rW^{2/3}} = s \times W^{1/3}.$$

Calculation of the United Risk from Animal Studies -- The risk associated with d mg/$kg^{2/3}$/day is obtained from GLOBAL79 and, for most cases of interest to risk assessment, can be adequately approximated by $P(d) = 1 - \exp(-q_1{*}d)$. A "unit risk" in units X is simply the risk corresponding to an exposure of X = 1. This value is estimated simply by finding the number of mg/$kg^{2/3}$/day that corresponds to one unit of X, and substituting this value into the above relationship. Note that an equivalent method of calculating unit risk would be to use mg/kg for the animal exposures, and then to increase the j^{th} polynomial coefficient by an amount

$$(W_h/W_a)^{j/3} \quad j = 1, 2, \ldots, k,$$

and to use mg/kg equivalents for the unit risk values.

Adjustments for Less Than Lifespan Duration of Experiment. If the duration of experiment L_e is less than the natural lifespan of the test animal L, the slope $q_1{*}$, or more generally the exponent g(d), is increased by multiplying a factor $(L/L_e)^3$. We assume that if the average dose d is continued, the age-specific rate of cancer will continue to increase as a constant function of the background rate. The age-specific rates for humans increase at least by the third power of the age and often by a considerably higher power, as demonstrated by Doll (1971). Thus, it is expected that the cumulative tumor rate would increase by at least the third power of age. Using this fact, it is assumed that the slope q_1*, or more generally the exponent g(d), would also increase by at least the third power of age. As a result, if the slope q_1* [or g(d)] is calculated at age L_e, it is expected that if the experiment had been continued for the full lifespan L at the given average exposure, the slope q_1* [or g(d)] would have been increased by at least $(L/L_e)^3$.

This adjustment is conceptually consistent with the proportional hazard model proposed by Cox (1972) and the time-to-tumor model considered by Daffer et al. (1980), where the probability of cancer by age t and at dose d is given by

$$P(d,t) = 1 - \exp [-f(t) \times g(d)].$$

Unit Risk Estimates --

Data Available for Potency Calculation. Hexachlorobenzene has been shown to induce tumors in hamsters, mice and rats. The primary target organ appears to be the liver in all three of these species. Liver haemangioendotheliomas in hamsters and hepatocellular carcinomas in rats were significantly increased in the hexachlorobenzene-treated animals. The potency estimate calculated on the basis of hepatocellular carcinomas in female rats is used to derive unit risk estimates for hexachlorobenzene in water. This particular tumor site is selected for calculating unit risks because it is a malignant tumor in the primary target organ and results in the highest potency estimate.

Increased incidences of thyroid, parathyroid, adrenal and kidney tumors were also observed among these species. Fourteen data sets showing significant tumor incidences have been used herein to calculate the carcinogenic potency of hexachlorobenzene. These calculations provide a range of estimates that, in part, reflect the uncertainties inherent in the risk assessment process. Tables VIII-2 through VIII-5 summarize the data used to calculate the potency of hexachlorobenzene.

Choice of Low-Dose Extrapolation -- In addition to the multistage model currently used by CAG for low-dose extrapolation, CAG also uses three other models, the probit, the Weibull and the one-hit models, to estimate the risks from exposure to hexachlorobenzene using the data for hepatocellular carcinoma in female rats. These models cover almost the entire spectrum of risk estimates that could be generated from the existing mathematical extrapolation models. These models are generally statistical in character, and are not derived from biological arguments, except for the multistage model which has been used to support the somatic mutation hypothesis of carcinogenesis (Armitage and Doll, 1954; Whittemore, 1978; Whittemore and Keller, 1978). The main differences among these models is the rate at which the response function, P(d), approaches zero or P(0) as dose, d, decreases. For instance, the probit model would usually predict a smaller risk at low doses than the multistage model because of the difference of the decreasing rate in the low-dose region. However, it should be noted that one could always artificially give the multistage model the same (or even greater)

TABLE VIII-2

Tumor Incidences in Male and Female Hamsters Given Hexachlorobenzene in Diet[a]

Dose[b] (mg/kg/day)	Thyroid	Hepatoma		Liver Hemangioendothelioma	
	Male	Male	Female	Male	Female
0	0/40	0/40	0/30	0/40	0/39
4	0/30	14/30	14/30	1/30	0/30
9	1/30	26/30	17/30	6/30	2/30
16	8/57	49/57	51/60	20/57	7/60

[a] Source: Cabral et al., 1977

[b] If mg/surface area/day is assumed to be equivalent between humans and animals, the dose in mg/kg/day is multiplied by a factor $(0.1/70)^{1/3}$, where 70 and 0.1 kg are, respectively, the average body weights of humans and hamsters.

TABLE VIII-3

Incidence of Liver Cell Tumors in Male and Female Swiss Mice Given Hexachlorobenzene Diet[a]

Dose[b] (mg/kg/day)	Male[c]	Female[c]
0	0/47	0/49
6	0/30	0/30
12	3/12	3/12
24	7/29	14/26

[a] Source: Cabral et al., 1979

[b] If the equivalent dose between humans and mice is assumed to be on the basis of body surface area, the dose in mg/kg/day is multiplied by a factor $(0.035/70)^{1/3}$, where 0.035 kg and 70 kg are, respectively, the average body weights of mice and humans.

[c] The number of animals that survived at the first observed liver cell tumor is used as the denominator.

TABLE VIII-4

Liver and Kidney Tumor Incidence Rates in Male and Female Sprague-Dawley Rats Given Hexachlorobenzene in Diet[a]

Sex	Dose[b] (mg/kg/day)	Hepatocellular Carcinoma	Hepatoma	Renal Cell Adenoma
Male	0	0/54	0/54	7/54
	4.24	3/52	10/52	41/52
	8.48	4/56	11/56	42/56
Female	0	0/52	0/52	1/52
	4.67	36/56	26/56	7/56
	9.34	48/55	35/55	15/54

[a] Source: Lambrecht, 1983a,b. Additional data from this study on adrenal pheochromocytoma has recently become available (Peters et al., 1983) but was not available when quantitative estimates were made.

[b] The dosages are calculated by the investigator based on the average food consumption of 22.6 g/rat/day and an average body weight of 400 g for male rats. For female rats, the average food consumption is 16.5 g/rat/day and the average body weight is 265 g. If the equivalent dose between humans and mice is assumed to be on the basis of body surface area, the dose presented in the table is multiplied by a factor $(W_a/70)^{1/3}$, where W_a is the body weight of male or female rats, and 70 kg is the human body weight.

TABLE VIII-5

Incidence Rate of Adrenal Pheochromocytoma in Female Sprague-Dawley Rats (F_1 generation) in a 2-Generation Feeding Study

Dose[a] (mg/kg/day)	Incidence Rate[b] (used in calculations)	Revised Incidence Rate[c]
0	2/48	2/49
0.02	4/50	4/49
0.08	4/50	
0.40	5/49	4/49
1.90	17/49	

[a] If the equivalent dose between humans and rats is assumed to be on the basis of body surface, the dose in this table is multiplied by a factor $(0.35/70)^{1/3}$, where 70 kg and 0.35 kg are, respectively, assumed to be the body weight of humans and rats.

[b] Source: Arnold et al., 1985

[c] Source: Arnold, 1984. The amended 1984 data were not available when quantitative estimates were made.

rate of decrease as the probit model by making some dose transformation and/or by assuming that some of the parameters in the multistage model are zero. This, of course, is not reasonable without knowing, a priori, what the carcinogenic process for the agent is. Although the multistage model appears to be the most reasonable or at least the most general model to use, the point estimate generated from this model is of limited value because it does not help to determine the shape of the dose-response curve beyond experimental exposure levels. Furthermore, point estimates at low doses extrapolated beyond experimental doses could be extremely unstable and could differ drastically, depending on the amount of the lowest experimental dose. Since upper-bound estimates from the multistage model at low doses are relatively more stable than point estimates, it is suggested that the upper-bound estimate for the risk (or the lower-bound estimate for the dose) be used in evaluating the carcinogenic potency of a suspect carcinogen. The upper-bound estimate can be taken as a plausible estimate if the true dose-response curve is actually linear at low doses. The upper-bound estimate means that the risks are not likely to be higher, but could be lower if the compound has a concave upward dose-response curve or a threshold at low doses. Another reason one can, at best, obtain an upper-bound estimate of the risk when animal data are used is that the estimated risk is a probability conditional to the assumption that an animal carcinogen is also a human carcinogen. Therefore, in reality, the actual risk could range from a value near zero to an upper-bound estimate.

__Calculation of the Carcinogenic Potency of Hexachlorobenzene__ -- Fourteen sets of tumor incidences which show significant increases (see Tables VIII-2 through VIII-5) are used herein to calculate the carcinogenic potency of hexachlorobenzene. Since preparing these calculations additional data from the Lambrecht et al. (1983a,b) study (adrenal pheochromocytoma) and from the Arnold et al. (1985) study (neoplastic liver nodules) have become available. Quantitative estimates have not been made using this data. Using the multistage model for low-dose extrapolation, as shown in Table VIII-6, the potency estimates calculated on the basis of these data sets are approximately within an order of magnitude from each other, with the exception of the thyroid tumor. These potencies provide a range of estimates that reflects the uncertainties stemming from the differences in species, tumor sites, solvent vehicles and composition of diet. The range does not reflect uncertainty resulting from the use of different extrapolation models.

To calculate the unit risks of hexachlorobenzene in water, CAG used an estimate of carcinogenic potency based upon the data for hepatocellular carcinoma in female rats. For comparison, three additional low-dose extrapolation models, the probit, the Weibull and the one-hit models, are also used to provide risk estimates at dose levels 0.01, 0.1 and 1 mg/kg/day. These results are presented in Table VIII-7. The maximum likelihood estimate of the parameters for all four models are presented in Table A-1 in the Appendix. At 1 mg/kg/day, all four models predict comparable risks. At lower doses, the multistage model predicts a higher risk than the probit model, but a lower risk than the Weibull model.

TABLE VIII-6

The Carcinogenic Potency[a] of Hexachlorobenzene, Calculated on the Basis of 14 Data Sets,[b] Using the Linearized Multistage Model

Study	Date Base	Dose is Assumed to be Equivalent on the Basis of		Reference
		Body Weight	Surface Area	
Hamster	Thyroid (male)	9.3×10^{-3}	8.3×10^{-2}	Cabral et al., 1977
	Hepatoma:			
	Male	1.9×10^{-1}	1.7	
	Female	1.5×10^{-1}	1.3	
	Hemangioendothelioma:			
	Male	3.2×10^{-2}	2.8×10^{-1}	
	Female	1.1×10^{-2}	1.0×10^{-1}	
Mice	Liver cell:			Cabral et al., 1979
	Male	1.7×10^{-2}	2.1×10^{-1}	
	Female	1.4×10^{-2}	1.8×10^{-1}	

TABLE VIII-6 (cont.)

Study	Date Base	Dose is Assumed to be Equivalent on the Basis of		Reference
		Body Weight	Surface Area	
Rats	Renal cell:			Lambrecht, 1983
	Male	2.5×10^{-1}	1.4	
	Female	4.2×10^{-2}	2.6×10^{-1}	
	Hepatocellular carcinoma:			
	Male	1.8×10^{-2}	1.0×10^{-1}	
	Female	2.7×10^{-1}	1.7	
	Hepatoma:			
	Male	4.7×10^{-2}	2.6×10^{-1}	
	Female	1.5×10^{-1}	9.0×10^{-1}	
Rats 2-generation study	Adrenal Pheochromocytoma (female)	2.8×10^{-1}	1.6	Arnold et al., 1983

[a] q_1^* (mg/kg/day)$^{-1}$ is the 95% upper confidence limit of the linear component in the multistage model.

[b] Since preparing these calculations, additional data from Lambrecht et al. (1983a,b) study (adrenal pheochromocytoma) and from Arnold et al. (1985) study (neoplastic liver nodules) has become available. These data have not been evaluated.

TABLE VIII-7

Upper-Bound[a] (Point) Estimation of Risk, Based on Hepatocellular Carcinoma in Female Rats[b]

Assumption of Human Equivalent Dose	Models	Risk at Dose Level (mg/kg/day)		
		0.01	0.1	1.00
On the basis of body weight	multistage	2.7×10^{-3} (2.2×10^{-3})	2.7×10^{-2} (2.2×10^{-2})	2.4×10^{-1} (2.0×10^{-1})
	probit	3.6×10^{-9} (1.3×10^{-10})	1.0×10^{-3} (8.9×10^{-5})	3.4×10^{-1} (1.2×10^{-1})
	Weibull	1.2×10^{-2} (2.5×10^{-3})	8.4×10^{-2} (2.5×10^{-2})	4.3×10^{-1} (2.2×10^{-1})
	one-hit	2.7×10^{-3} (2.2×10^{-3})	2.7×10^{-2} (2.2×10^{-2})	2.4×10^{-1} (2.0×10^{-1})
On the basis of surface area	multistage	1.7×10^{-2} (1.4×10^{-2})	1.7×10^{-1} (1.3×10^{-1})	8.0×10^{-1} (7.4×10^{-1})
	probit	6.2×10^{-5} (4.1×10^{-6})	1.3×10^{-1} (2.9×10^{-2})	8.2×10^{-1} (7.5×10^{-1})
	Weibull	5.0×10^{-2} (1.3×10^{-2})	2.9×10^{-1} (1.3×10^{-1})	8.1×10^{-1} (7.4×10^{-1})
	one-hit	1.7×10^{-2} (1.4×10^{-2})	1.7×10^{-1} (1.3×10^{-1})	8.0×10^{-1} (7.4×10^{-1})

[a] 95% upper confidence limit

[b] Source: Lambrecht, 1983

Risk Associated with 1 μg/ℓ of Hexachlorobenzene in Drinking Water -- Under the assumption that daily water consumption for a 70 kg person is 2 ℓ, the hexachlorobenzene intake in terms of mg/kg/day is

$$d = 2\ \ell \times 1\ \mu g/\ell \times 10^{-3}\ mg/\ell/70\ kg = 2.86 \times 10^{-5}\ mg/kg/day.$$

Therefore, the risk from drinking water containing 1 μg/ℓ of hexachlorobenzene is estimated to be

$$P = 1.7 \times 2.86 \times 10^{-5} = 4.9 \times 10^{-5}.$$

This calculation uses the carcinogenic potency $q_1^* = 1.7/(mg/kg/day)$, based on the data on hepatocellular carcinomas in female rats, assuming that dose per surface area is equivalent between rats and humans. If the equivalent dose is assumed to be on the basis of body weight, the unit risk, P, would be reduced to 7.6×10^{-6}.

Comparison of Potency with Other Compounds. One of the uses of quantitative potency estimates is to compare the relative potency of carcinogens. Figure VIII-1 is a histogram representing the frequency distribution of potency indices for 54 suspect carcinogens evaluated by CAG. The actual data summarized by the histogram are presented in Table VIII-8. The potency index is derived from q_1^*, the 95% upper bound of the linear component in the multistage model, and is expressed in terms of $(mMol/kg/day)^{-1}$. Where no human data were available, animal oral studies were used in preference to animal inhalation studies, since oral studies have constituted the majority of animal studies.

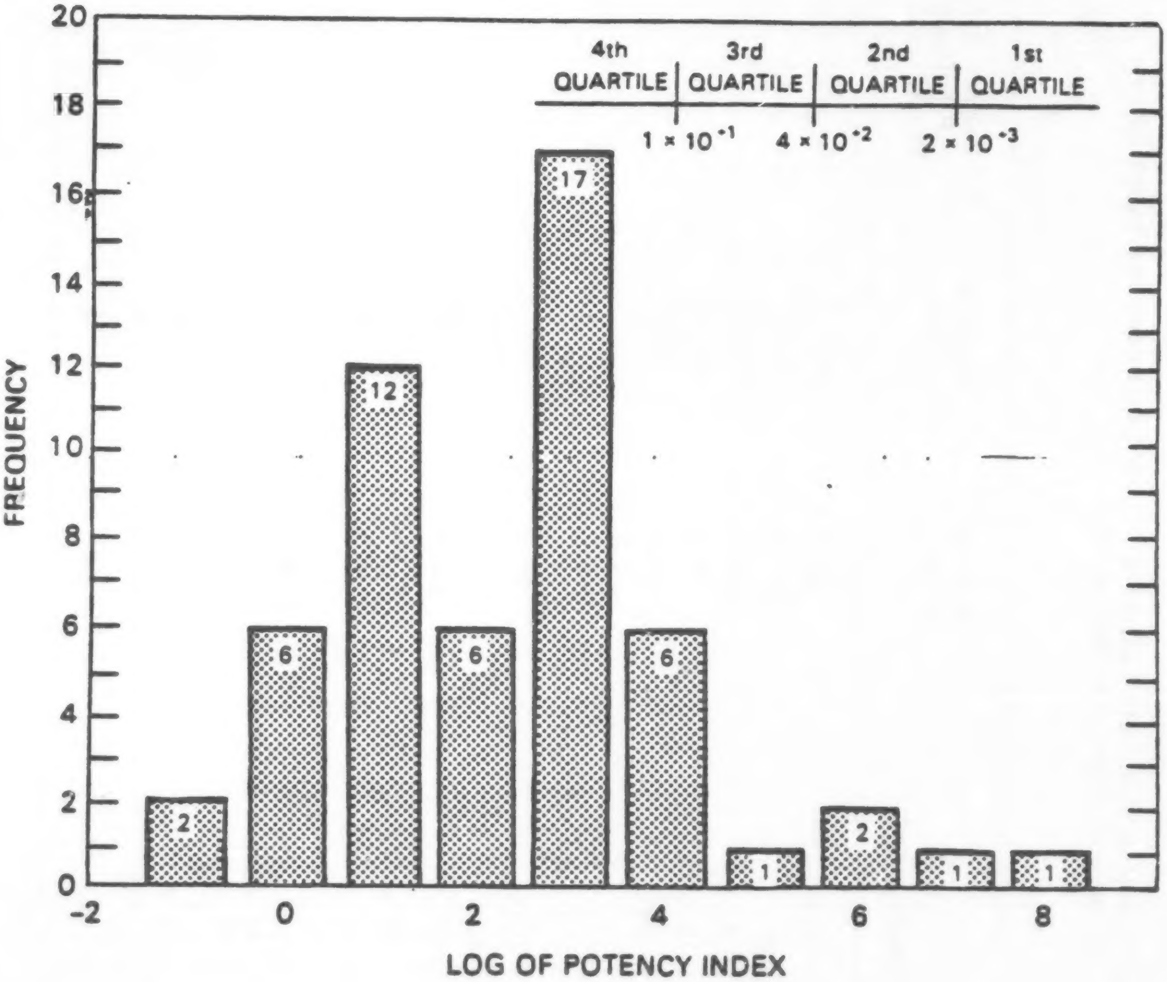

FIGURE VIII-1

Histogram Representing the Frequency Distribution of the Potency Indices of 54 Suspect Carcinogens Evaluated by the Carcinogen Assessment Group

TABLE VIII-8

Relative Carcinogenic Potencies Among 54 Chemicals Evaluated by the Carcinogen Assessment Group as Suspect Human Carcinogens[a,b,c]

Compounds	Slope (mg/kg/day)$^{-1}$	Molecular Weight	Potency Index	Order of Magnitude ($\log_{10}$ index)
Acrylonitrile	0.24 (W)	53.1	$1\times10^{+1}$	+1
Aflatoxin B_1	2924	312.3	$9\times10^{+5}$	+6
Aldrin	11.4	369.4	$4\times10^{+3}$	+4
Allyl chloride	1.19×10^{-2}	76.5	9×10^{-1}	0
Arsenic	15 (H)	149.8	$2\times10^{+3}$	+3
B[a]P	11.5	252.3	$3\times10^{+3}$	+3
Benzene	5.2×10^{-2} (W)	78	4×10^{0}	+1
Benzidene	234 (W)	184.2	$4\times10^{+4}$	+5
Beryllium	1.40	9	$1\times10^{+1}$	+1
Cadmium	6.65 (W)	112.4	$7\times10^{+2}$	+3
Carbon tetrachloride	$1,30 \times 10^{-1}$	153.8	$2\times10^{+1}$	+1
Chlordane	1.61	409.8	$7\times10^{+2}$	+3
Chlorinated ethanes				
1,2-Dichloroethane	6.9×10^{-2}	98.9	7×10^{0}	+1
Hexachloroethane	1.42×10^{-2}	236.7	3×10^{0}	0
1,1,2,2-Tetrachloroethane	0.20	167.9	$3\times10^{+1}$	+1
1,1,1-Trichloroethane	1.6×10^{-3}	133.4	2×10^{-1}	-1
1,1,2-Trichloroethane	5.73×10^{-2}	133.4	8×10^{0}	+1
Chloroform	7×10^{-2}	119.4	8×10^{0}	+1
Chromium	41 (W)	100	$4\times10^{+3}$	+4

TABLE VIII-8 (cont.)

Compounds	Slope (mg/kg/day)$^{-1}$	Molecular Weight	Potency Index	Order of Magnitude ($\log_{10}$ index)
DDT	8.42	354.5	$3\times10^{+3}$	+3
Dichlorobenzidine	1.69	253.1	$4\times10^{+2}$	+3
1,1-Dichloroethylene	1.47×10^{-1} (I)	97	$1\times10^{+1}$	+1
Dieldrin	30.4	380.9	$1\times10^{+4}$	+4
Dinitrotoluene	0.31	182	$6\times10^{+1}$	+2
Diphenylhydrazine	0.77	180	$1\times10^{+2}$	+2
Epichlorohydrin	9.9×10^{-3}	92.5	9×10^{-1}	0
Bis(2-chloroethyl)ether	1.14	143	$2\times10^{+2}$	+2
Bis(chloromethyl)ether	9300 (I)	115	$1\times10^{+6}$	+6
Ethylene dibromide (ECB)	8.51	187.9	$2\times10^{+3}$	+3
Ethylene oxide	1.26 (I)	44.1	$6\times10^{+1}$	+2
Heptachlor	3.37	373.3	$1\times10^{+3}$	+3
Hexachlorobenzene	1.67	284.4	$5\times10^{+2}$	+3
Hexachlorobutadiene	7.75×10^{-2}	261	$2\times10^{+1}$	+1
Hexachlorocyclohexane				
Technical grade	4.75	290.9	$1\times10^{+3}$	+3
Alpha isomer	11.12	290.9	$3\times10^{+3}$	+3
Beta isomer	1.84	290.9	$5\times10^{+2}$	+3
Gamma isomer	1.33	290.9	$4\times10^{+2}$	+3
Hexachlorodibenzodioxin	$1.1 \times 10^{+4}$	391	$4\times10^{+6}$	+7
Methylene chloride	6.3×10^{-4}	84.9	5×10^{-2}	-1
Nickel	1.15 (W)	58.7	$7\times10^{+1}$	+2

TABLE VIII-8 (cont.)

Compounds	Slope (mg/kg/day)$^{-1}$	Molecular Weight	Potency Index	Order of Magnitude (log$_{10}$ index)
Nitrosamines				
Dimethylnitrosamine	25.9 (not by q_1*)	74.1	2x10^{+3}	+3
Diethylnitrosamine	43.5 (not by q_1*)	102.1	4x10^{+3}	+4
Dibutylnitrosamine	5.43	158.2	9x10^{+2}	+3
N-nitrosopyrrolidine	2.13	100.2	2x10^{+2}	+2
N-nitroso-N-ethylurea	32.9	117.1	4x10^{+3}	+4
N-nitroso-N-methylurea	302.6	103.1	3x10^{+4}	+4
N-nitroso-diphenylamine	4.92 x 10^{-3}	198	1x10^{0}	0
PCBs	4.34	324	1x10^{+3}	+3
Phenols				
2,4,6-Trichlorophenol	1.99 x 10^{-2}	197.4	4x10^{0}	+1
Tetrachlorodibenzo-p-dioxin	1.56 x 10^{5}	322	5x10^{+7}	+8
Tetrachloroethylene	3.5 x 10^{-2}	165.8	6x10^{0}	+1
Toxaphene	1.13	414	5x10^{+2}	+3
Trichloroethylene	1.9 x 10^{-2}	131.4	2.5x10^{0}	0
Vinyl chloride	1.75 x 10^{-2} (I)	62.5	1x10^{0}	0

[a] Animal slopes are 95% upper-limit slopes based on the linearized multistage model. They are calculated based on animal oral studies, except for those indicated by I (animal inhalation), W (human occupational exposure, and H (human drinking water exposure). Human slopes are point estimates based on the linear non-threshold model.

[b] The potency index is a rounded-off slope in (mMol/kg/day)$^{-1}$ and is calculated by multiplying the slopes in (mg/kg/day)$^{-1}$ by the molecular weight of the compound.

[c] Not all of the carcinogenic potencies presented in this table represent the same degree of certainty. All are subject to change as new evidence becomes available.

Based on data concerning hepatocellular carcinomas in female rats, the potency index for hexachlorobenzene has been calculated as 5×10^2. This figure is derived by multiplying the slope $q_1^* = 1.7/(mg/kg/day)$ and the molecular weight of hexachlorobenzene, 284.4. This places the potency index for hexachlorobenzene in the second quartile of the 54 suspect carcinogens evaluated by CAG.

The ranking of relative potency indices is subject to the uncertainties involved in comparing a number of potency estimates for different chemicals based on varying routes of exposure in different species by means of data from studies whose quality varies widely. All of the indices presented are based on estimates of low-dose risk, using linear extrapolation from the observational range. These indices may not be appropriate for the comparison of potencies if linearity does not exist at the low-dose range, or if comparison is to be made at the high-dose range. If the latter is the case, then an index other than the one calculated above may be more appropriate.

Summary of Quantitative Estimation. Data on hepatocellular carcinomas in female rats after oral ingestion have been used to estimate the carcinogenic potency of hexachlorobenzene and the risks associated with one unit of the compound in drinking water. The upper bound cancer risks associated with 1 μg/ℓ of hexachlorobenzene in drinking water is estimated to be 4.9×10^{-5}. Accordingly, upper bound cancer risks of 10^{-6}, 10^{-5} and 10^{-4} would be associated with 0.02, 0.2 and 2 μg/ℓ, respectively, of hexachlorobenzene in drinking water. This estimate is calculated on the basis of the assumption that dose per surface area is equivalent among species. If the dose is assumed to be equivalent on the basis of body weight, the corresponding risk would be reduced approximately by a factor of 6. The carcinogenic potencies of hexachlorobenzene are also estimated on the basis of 13 other data sets, encompassing different tumor sites and animal species. Except for the case of thyroid tumors, these potency estimates differ from each other within a single order of magnitude. The range of the estimates reflects the uncertainties due to differences in species, tumor sites, solvent vehicles, composition of diet, etc.

High Risk Subpopulations

Infants and children (4-16 years of age) appear to be the most susceptible to exposure to hexachlorobenzene. This susceptibility was observed after the accidental ingestion of hexachlorobenzene-contaminated grain occurred in Turkey during 1955-1959 (Peters et al., 1982). A distinct disease described as "Pink Sore", which reached an epidemic scale, was observed in infants under 1 year of age. These infants' mothers had consumed the hexachlorobenzene-treated grain during gestation and/or lactation. This exposure has also been associated with the observed 95% 1-year-old infant mortality and the high incidence of stillbirths. During the same Turkish accident, children (4-16 years of age) were found to be more susceptible to porphyria cutanea tarda than the adults.

There are strong indications that adult females may be more susceptible to hexachlorobenzene-induced porphyria than adult males, which is the case in the rodent studies (Rizzardini and Smith, 1982). According to these researchers, this increased susceptibility in adult females is related to their estrogen levels and faster metabolism of hexachlorobenzene.

Summary

Health advisories based on noncarcinogenic toxicity data and the carcinogenic risk assessment are given in Table VIII-9. Available data are concluded to be insufficient for calculation of a 1-day HA. The 10-day HA, of 0.175 mg/ℓ for a 70 kg adult and 0.050 mg/ℓ for a 10 kg child, which is also being proposed as the 1-day HA, is based on the study by Kuiper-Goodman et al. (1977) in which male and female Charles River rats were fed hexachlorobenzene in the diet for as long as 15 weeks. An AADI of 0.028 mg/ℓ for a 70 kg adult is based on noncarcinogenic effects in male and female Sprague-Dawley rats exposed to hexachlorobenzene _in utero_, during lactation, and in the diet for the remainder of their lifetimes. However, there is sufficient evidence for the carcinogenicity of hexachlorobenzene in experimental animals (IARC, 1979). By using the 95% upper limit with the multistage model and the hepatocellular carcinoma data in female rats from the Lambrecht et al. (1983) hexachlorobenzene carcinogenicity study, lifetime exposures to hexachlorobenzene in drinking water at levels of 2×10^{-5}, 2×10^{-4} and 2×10^{-3} mg/ℓ are associated with 10^{-6}, 10^{-5} and 10^{-4} cancer risks, respectively.

TABLE VIII-9

Summary of the Data for Hexachlorobenzene Used to Derive HA and AADI

Health Advisory	Species/Route	Dose (mg/kg bw/day)	Duration	Basis	Uncertainty Factors	Value[a] (mg/ℓ)	Reference
1-Day and 10-Day	rat/oral	0.5	15 weeks	NOAEL, higher doses cause an increase in liver, kidney and spleen porphyrin levels and centrilobular liver lesions	100	0.175[b] 0.050[c]	Kuiper-Goodman et al., 1977
AADI	rat/oral	0.08	130 weeks	NOAEL, higher doses cause an increase in liver and kidney lesions	100	0.028[b]	Arnold et al., 1983
Cancer number	rat/oral	"linearized" multistage model	lifetime	Hepatocellular carcinomas	NA	2.0×10^{-5}[b,d]	Lambrecht et al., 1983

[a] Hexachlorobenzene water solubility is referenced as 0.005 mg/ℓ @ 25°C

[b] For a 70 kg adult

[c] For a 10 kg child

[d] Water concentration associated with an upper level excess lifetime cancer risk of 10^{-6}. Values of 2×10^{-4} and 2×10^{-3} mg/ℓ are associated with upper level excess lifetime cancer risks of 10^{-5} and 10^{-4}, respectively.

NA = Not applicable

IX. REFERENCES

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